FACTORS AFFECTING UTILIZATION OF PHOSPHORUS BY POULTRY

By

PARK WILLIAM WALDROUP

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS i

LIST OF TABLES v

LIST OF FIGURES viii

CHAPTER

1 INTRODUCTION 1

2 MATERIALS AND METHODS 4

3 THE INFLUENCE OF PHOSPHORUS LEVELS IN THE MATERNAL DIET 6

Experimental Procedure 6
Results and Discussion 9

4 COMPARISON OF THE REQUIREMENTS OF BATTERY AND FLOOR GROWN CHICKS FOR CALCIUM AND PHOSPHORUS 14

Experimental Procedure 14
Results and Discussion 16

5 A COMPARISON OF PHOSPHORUS ASSAY TECHNIQUES WITH CHICKS 21

Experimental Procedure 22
Results and Discussion 24

6 THE UTILIZATION OF VARIOUS SOURCES OF CALCIUM . . . 29

Experimental Procedure 30
Trial 1 30
Trial 2 33

Results and Discussion 33
Trial 1 33
Trial 2 36

TABLE OF CONTENTS--Continued

CHAPTER Page

7 THE EFFECT OF VITAMIN D ON PHOSPHORUS UTILIZATION . . 39

The Interaction of Calcium, Phosphorus, and Vitamin D 39
  Experimental Procedure 39
  Results and Discussion 42

The Vitamin D Requirement of the Chick as Influenced by the Dietary Calcium and Phosphorus Level 55
  Experimental Procedure 55
    Battery brooder studies 55
    Floor pen studies 56
  Results and Discussion 58
    Battery brooder studies 58
    Floor pen studies 61

8 THE AVAILABILITY OF PHOSPHORUS FROM ANIMAL PROTEIN SOURCES 67

  Experimental Procedure 67
  Results and Discussion 71

9 THE AVAILABILITY OF PHOSPHORUS FROM PLANT SOURCES . . 74

  The Availability of Phytic Acid Phosphorus for Chicks 76
    Experimental Procedure 76
    Results and Discussion 78

  Comparison of Phytin Phosphorus Sources 80
    Experimental Procedure 80
      Trial 1 81
      Trial 2 83
      Trial 3 83
    Results and Discussion 84
      Trial 1 84
      Trial 2 84
      Trial 3 86

  Effect of Calcium and Vitamin D_3 Levels on the Utilization of Calcium Phytate 91
    Experimental Procedure 91
      Trial 1 92
      Trial 2 93

TABLE OF CONTENTS--*Continued*

CHAPTER Page

9 Results and Discussion 93
Trial 1 93
Trial 2 97

The Availability of Natural Plant Phosphorus . . . 100
Experimental Procedure 100
Results and Discussion 104

10 SUMMARY AND CONCLUSIONS 109

Phosphorus Levels in the Maternal Diet 109
Variation Between Battery and Floor Conditions . . 110
Comparison of Assay Techniques 110
Source of Calcium 110
Vitamin D Levels 110
Availability of Phosphorus from Animal Protein Supplements 111
Availability of Phosphorus from Plant Sources . . 111

REFERENCES 113

LIST OF TABLES

Table		Page
1	Composition of diets	7
2	Hatchability and phosphorus and calcium content of eggs when hens were fed diets with and without supplemental inorganic phosphorus	10
3	Ash, phosphorus, and calcium content of the tibia from day-old chicks hatched from hens fed diets with and without supplemental inorganic phosphorus	12
4	Ash, phosphorus and calcium content of the tibia from two week old chicks hatched from hens fed diets with and without supplemental inorganic phosphorus	13
5	Composition of basal diet	15
6	Body weight and feed efficiency of chicks grown in batteries and floor pens when fed diets containing various levels of phosphorus and calcium (Trial 1)	17
7	Body weight and bone ash of chicks grown in batteries and floor pens when fed diets containing various levels of phosphorus and calcium (Trial 2)	18
8	Composition of basal diet	23
9	Body weight and tibia ash of 28-day old chicks fed varying levels of phosphorus from two sources under three regimens of calcium supplementation	25
10	Composition of basal diet	31
11	Analysis of calcium supplements	32
12	Bone ash of chicks grown on diets with different calcium levels from various calcium supplements (Trial 1)	34

LIST OF TABLES--*Continued*

Table Page

13 Body weights of chicks grown on diets with different calcium levels from various calcium supplements (Trial 1) 35

14 Body weight of chicks fed diets with different calcium levels from various calcium supplements (Trial 2) 37

15 Tibia ash of chicks fed diets with different calcium levels from various calcium supplements (Trial 2) . . 38

16 Composition of basal diet 41

17 Body weight of chicks grown on diets varying in levels of calcium, phosphorus, and vitamin D_3 43

18 Percent of bone ash of chicks grown on diets varying in levels of calcium, phosphorus, and vitamin D_3 . . 44

19 Composition of diets 57

20 Body weight of chicks fed different levels of vitamin D_3 at different calcium and phosphorus levels (battery brooder studies) 59

21 Tibia ash of chicks fed different levels of vitamin D_3 at different calcium and phosphorus levels (battery brooder studies) 60

22 Body weight of chicks fed different levels of vitamin D_3 at different dietary calcium levels (floor pen study) 62

23 Tibia ash of chicks fed different levels of vitamin D_3 at different dietary calcium levels (floor pen study) 63

24 Feed utilization of chicks fed different levels of vitamin D_3 at different dietary calcium levels (floor pen study) 64

25 Analyses of animal protein sources 68

26 Composition of basal diets 70

LIST OF TABLES--Continued

Table Page

27 Body weight of chicks fed diets supplemented with phosphorus from animal proteins and inorganic phosphate 72

28 Tibia ash of chicks fed diets supplemented with phosphorus from animal proteins and inorganic phosphate 73

29 Composition of basal diets 77

30 Body weight and tibia ash of chicks fed various levels of phosphorus from phytic acid and dicalcium phosphate 79

31 Composition of basal diet 82

32 Body weight and tibia ash of chicks fed diets supplemented with various sources of organic and inorganic phosphorus 85

33 Body weight of chicks fed diets supplemented with various sources of organic and inorganic phosphorus . 87

34 Body weight of chicks fed diets supplemented with various sources of organic and inorganic phosphorus . 88

35 Body weight of chicks fed different phosphorus sources, levels of vitamin D_3, and calcium:phosphorus ratios . 94

36 Tibia ash of chicks fed different phosphorus sources, vitamin D_3 levels, and calcium:phosphorus ratios . . 95

37 Body weight of chicks fed different phosphorus sources with varying levels of calcium and vitamin D_3 98

38 Phosphorus analysis of corn products 101

39 Composition of diets 102

40 Body weight of broiler chicks fed diets with plant phosphorus from three sources 105

41 Tibia ash of broiler chicks fed diets with plant phosphorus from three sources 107

LIST OF FIGURES

Figure — Page

1 Bone ash and body weight of chicks receiving various dietary phosphorus levels 47

2 Bone ash and body weight of chicks receiving various calcium:phosphorus ratios 49

3 Bone ash and body weight of chicks receiving various dietary levels of vitamin D_3 50

4 Bone ash of chicks receiving various Ca:P ratios with various phosphorus levels 51

5 Bone ash of chicks receiving various levels of vitamin D_3 with various calcium:phosphorus ratios . 52

6 Bone ash of chicks receiving various levels of vitamin D_3 with various phosphorus levels 54

CHAPTER 1

INTRODUCTION

The element phosphorus probably plays a more varied and important role in the chemistry of living organisms than any other single element. It was first prepared in the free state in 1669 by Brandt, a German chemist, and first recognized as an essential constituent of bones by Gahn, a Swedish chemist, in 1769. Subsequent research has demonstrated that it is also an essential constituent of proteins and fats occurring in muscular tissues and vital organs. Phosphorus has, by virtue of its association with nucleic acids, been found to be an important part of the structure of chromosomes. Phosphates are also known to be important buffers in tissue fluids.

That animal rations may sometimes be deficient in mineral elements began to be recognized in France and Germany about 100 years ago when weak bones in cattle grazing in certain localities began to be associated with mineral deficiencies in the soil. Ewing (1963), in tracing the history of phosphorus in animal feeding, stated that in 1861 Van Gohren reported that the ocurrence of weak bones in cows grazing in certain areas near the Rhine River could be prevented and cured by feeding small amounts of bone meal. Subsequent analyses of the soil and grass in these areas revealed an abnormally low percentage of phosphorus and, to a lesser extent, calcium. This report is the earliest recorded use of a phosphate feed supplement for the specific purpose of preventing a phosphorus deficiency disease in livestock, according to this reviewer.

The most abundant source of phosphorus for feed purposes is rock phosphate. World reserves of 26 billion tons have been estimated, about half of which is located in the United States. It began to be used as a source of phosphorus in plant nutrition as early as 1860, and fed to livestock in the United States about 1915. The danger of fluorine toxicity in raw rock phosphate limited its use until 1940, when defluorinated superphosphate was first produced on a commercial scale. Many types of phosphates are now in use in animal feeds.

Numerous studies have been conducted to establish the phosphorus requirement of the chick, with quite a variation in reported requirements. McGinnis _et_ _al._ (1944) reported that levels of greater than 0.58 percent phosphorus were required for maximum calcification. Singsen _et_ _al._ (1947) concluded that the available phosphorus requirement for satisfactory calcification appeared to lie between 0.38 and 0.47 percent of the diet. Gillis _et_ _al._ (1949) reported a requirement of approximately 0.50 percent. This level was also suggested by O'Rourke _et_ _al._ (1952). Grau and Zweigart (1953) indicated that maximum tibia ash of chicks was obtained with a level of not more than 0.45 percent phosphorus.

Fisher _et_ _al._ (1953) reported that chicks required 0.58 percent total phosphorus for maximum calcification. Couch _et_ _al._ (1937) suggested that a level of 0.76 to 0.81 percent phosphorus was adequate for normal growth and bone calcification of chicks up to twelve weeks of age. Further studies are reviewed by Singsen et al. (1948), Gillis et al. (1949) O'Rourke et al. (1952), and Nelson and Walker (1964).

A standard assay method for evaluating phosphorus compounds is desirable in order to establish uniform values for each supplement. However, considerable variation has been observed in the methods used to test the utilization of the various phosphates. In certain assays constant calcium to phosphorus ratios were employed (Creech et al., 1956, Nelson and Peeler, 1961), while in others constant calcium levels of 1.0 percent (Ammerman et al., 1960) or 1.2 percent (Gardiner et al., 1959) were used. Similar variation has been observed in vitamin D levels, supplementary phosphorus level, reference phosphate used, and other factors.

The variability in these reports indicates the necessity for a standard assay procedure for determining both the requirement of the chick for phosphorus and the availability of phosphate supplements. Therefore, studies were undertaken to determine factors which may account for variation in phosphorus utilization by the chick in an attempt to develop a standard assay procedure which would permit a more accurate study of phosphorus in poultry diets.

CHAPTER 2

MATERIALS AND METHODS

The broiler chicks used in these studies were a Vantress x White Plymouth Rock cross obtained from a commercial hatchery. At one day of age the chicks were sexed, debeaked, vaccinated for Newcastle disease and infectious bronchitis, and randomly assigned to treatment pens.

The battery brooders used in these studies were Oakes 801-A five deck thermostatically controlled electrically heated battery brooders with raised wire floors.

Two types of floor pen facilities were used. The first type had pens 10 x 12 feet, equipped with 2 feed troughs, 4 feet in length, and 1 bell-type automatic water fount. The second type had pens that were 5 x 5 feet in size, each containing a tube-type feeder and a bell-type automatic water fount. Infrared bulbs were used as the heat source in both types of floor pens.

Chicks used for bone ash determinations were sacrificed at the selected age and the right tibia removed. The bones were boiled for 3 minutes, cleaned of adhering tissue, and lightly polished with cheesecloth. After drying for 24 hours at 100° C the bones were collectively ether extracted and then ashed individually.

The data collected in these studies were subjected to the analysis of variance as outlined by Snedecor (1957) with significant differences between treatment means determined by use of the multiple range test of Duncan (1955). Orthogonal components of variance were determined following the procedure of Snedecor (1957).

CHAPTER 3

THE INFLUENCE OF PHOSPHORUS LEVELS IN THE MATERNAL DIET

Assay methods for certain nutrients in the diet of chicks have been complicated to an extent by maternal influences. Variation in the amount of the nutrient supplied in the diet of the dam often influences the amount of this nutrient supplied in the egg to the developing embryo. This is true for certain vitamins and antibiotics and may also be true with inorganic nutrients. Therefore, variation in the phosphorus level of the maternal diet could possibly affect the results of phosphorus studies with young chicks, since most phosphorus studies are conducted with young chicks 3 to 4 weeks of age.

The objectives of this study were to determine the effect of the phosphorus level in the breeder diet upon (1) the hatchability of eggs (2) the mineral composition of the whole egg and the tibia of the day-old chicks, and (3) the rate of bone mineralization of the growing chick.

Experimental Procedure

Two groups, each containing 5 commercial egg production type hens, were fed on each of the basal diets shown in Table 1. Two additional groups of 5 hens were fed each of the basal diets supplemented with 0.35 percent phosphorus from feed grade defluorinated

TABLE 1

Composition of diets

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
	(Percent of diet)			
Yellow corn	37.10	32.10	65.60	59.90
Oats	30.00	29.20	. .	. .
Animal fat	. .	4.70	. .	4.70
Soybean meal (50% protein)	21.00	22.10	22.50	23.50
Alfalfa meal (17% protein)	5.00	5.00	5.00	5.00
Ground limestone	6.00	6.00	6.00	6.00
Iodized salt	0.40	0.40	0.40	0.40
Micro-ingredients[1]	0.50	0.50	0.50	0.50
% Total P	0.39	0.39	0.34	0.34

[1] Supplies per pound of feed: 2,000 I.U. vitamin A, 700 I.C.U. vitamin D_3, 6 mcg. vitamin B_{12}, 2 mg. riboflavin, 4 mg. calcium pantothenate, 6 mg. niacin, 227 mg. choline chloride, 2.5 I.U. vitamin E, 0.08 gms. $MnSO_4$ and 0.5 mg. menadione sodium bisulfite.

phosphate. The hens were maintained in individual wire cages and given the experimental feeds and tap water ad libitum. Since the composition of the diet did not affect hen performance or chick measurements, only the effect of supplemental phosphorus will be discussed.

After the hens had received these diets for 7 months they were inseminated twice each week with pooled semen from cockerels receiving a complete breeder diet. All eggs produced for a 9-day-period were incubated in order to determine hatchability. Two chicks from each hen were sacrificed at 1 day of age and the right tibia removed for bone ash, calcium, and phosphorus determination.

All remaining chicks were wingbanded and placed in electrically heated battery brooders and were fed the basal diet of Ammerman et al. (1961) with 0.2 percent supplemental phosphorus from defluorinated phosphate. They were given the experimental diet and tap water ad libitum. At 2 weeks of age all chicks were sacrificed and the right tibia removed for bone ash, phosphorus, and calcium determination.

The total ash, phosphorus and calcium content of the fresh egg were determined on 3 eggs from each hen immediately following the collection period for the hatchability study. The eggs were crushed and placed in a beaker, and the major portion of the moisture was removed in a drying oven before they were transferred to a muffle furnace for ashing.

The ash, calcium and phosphorus content of bones and eggs was determined by the procedures outlined by A.O.A.C. (1960).

Results and Discussion

Supplementing the basal diets used in this experiment with 0.35 percent phosphorus, from defluorinated phosphate, significantly increased hatchability of fertile eggs (Table 2). Although hatchability of eggs was increased by the supplemental phosphorus, it did not change the ash, phosphorus or calcium content of the fresh egg. The observation that diets low in phosphorus resulted in low hatchability without altering the percentage of phosphorus in the egg agrees with data reported by O'Rourke *et al.* (1954).

The finding that 0.35 or 0.39 percent total phosphorus was inadequate for normal hatchability does not agree with data of O'Rourke *et al.* (1954), who found a phosphorus level of 0.30 or 0.35 percent was adequate for normal hatchability. This discrepancy can be partially explained by the fact that the basal diet of O'Rourke *et al.* (1954) contained only 0.19 percent total phosphorus, therefore, it contained considerable supplemental inorganic phosphorus. The basal diet employed in this study contained no supplemental inorganic phosphorus. Since it is known that form of dietary phosphorus (organic vs. inorganic) greatly influences its availability, the difference in the type of phosphorus in the 2 basal diets could account for the difference in results.

The ash content of the tibia or the phosphorus or calcium content of the tibia of day-old or 14-day-old chicks was not influenced by the supplemental phosphorus in the maternal diet (Tables 3

TABLE 2

Hatchability and phosphorus and calcium content of eggs when hens were fed diets with and without supplemental inorganic phosphorus

% Supplemental Phosphorus	% Hatchability	Egg Content[1]			
		% Ash	% P	% Ca	No. Eggs
0	47.1	8.41	0.191	3.48	120
0.35	68.7[2]	8.67	0.189	3.56	120

[1] Expressed as a percentage of the fresh egg weight.

[2] This difference is statistically significant at the 0.01% level of probability.

and 4). The variation in tibia ash content from chicks within the same hen group was as great as the variation in bone ash values for chicks from different hens. Therefore, there would be no advantage in selecting chicks from certain hens for phosphorus assays in an attempt to decrease experimental error.

Supplemental phosphorus in the maternal diet did not appear to alter the chick's ability to use calcium and phosphorus for mineralization of the bone. Therefore, consideration of the phosphorus level of the maternal diet appears to be of limited value in the selection of the chicks for the biological assay of phosphate.

TABLE 3

Ash, phosphorus, and calcium content of the tibia from day-old chicks hatched from hens fed diets with and without supplemental inorganic phosphorus

% Supplemental Phosphorus	Tibia Content			No. Analyzed
	% Ash[1]	% P[2]	% Ca[2]	
0	34.38	16.79	15.55	80
0.35	34.32	15.41	14.53	80

[1] Expressed as a percentage of bone on a dry, fat-free basis.

[2] Expressed as a percentage of the tibia ash.

TABLE 4

Ash, phosphorus and calcium content of the tibia from two week old chicks hatched from hens fed diets with and without supplemental inorganic phosphorus

% Supplemental Phosphorus	Tibia Content			No. Analyzed
	% Ash[1]	% P[2]	% Ca[2]	
0	40.81	19.00	41.98	59
0.35	41.56	18.37	39.25	62

[1] Expressed as a percentage of bone on a dry, fat-free basis.

[2] Expressed as a percentage of the tibia ash.

CHAPTER 4

COMPARISON OF THE REQUIREMENTS OF BATTERY AND FLOOR GROWN CHICKS FOR CALCIUM AND PHOSPHORUS

It has recently been reported that the phosphorus requirement is higher for laying hens maintained in cages than for those maintained on litter in floor pens (Crowley *et al.*, 1961; Harms *et al.*, 1961; Marr *et al.*, 1961; and Singsen *et al.*, 1961). It has been suggested that hens on litter may be getting phosphorus from eating feces.

The calcium and phosphorus requirements of chicks were established primarily from experiments carried out in batteries with raised wire floors. Therefore, in view of the above mentioned reports it seemed desirable to determine whether this difference in requirements existed for battery and floor grown chicks.

Experimental Procedure

Two trials were conducted. In each trial day-old broiler chicks were randomized into 30 groups of 5 males and 5 females, and 20 groups of 50 males and 50 females. The 30 groups were randomly assigned to electrically heated battery pens, with the 20 groups assigned to 10 x 12 foot pens with wood shavings used for litter.

The basal diet (Table 5) contained 22 percent protein, 1,000 Calories of productive energy per pound, 0.60 percent calcium and 0.42

TABLE 5

Composition of basal diet

Ingredients	Percent of diet
Yellow corn	59.08
Soybean meal (50% protein)	32.45
Animal fat	2.90
Alfalfa meal (17% protein)	3.00
Dicalcium phosphate	0.42
Ground limestone	0.85
Iodized salt	0.40
Micro-ingredients[1]	0.90

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[1] Supplies per pound of feed: 2268 I.U. vitamin A, 10 mcg. vitamin B_{12}, 340 I.C.U. vitamin D_3, 2 mg. riboflavin, 9 mg. calcium pantothenate, 18 mg. niacin, 261 mg. choline chloride, 10 mg. terramycin Hcl, 57 mg. santoquin, 80 mg. manganese sulfate, 35 mg. manganous oxide, 9 mg. iron, 0.9 mg. copper, 90 mcg. cobalt, 5 mg. iodine, 45 mcg. zinc, with 25 mg. nitrofurazane and 3.6 mg. furazolidone added to feed for chicks on floor.

percent total phosphorus. This diet was modified to form 9 other experimental diets containing various levels of phosphorus and calcium as shown in Tables 6 and 7. The desired levels of calcium and phosphorus were attained by varying the amount of ground limestone and dicalcium phosphate. Experimental diets were maintained iso-caloric and iso-nitrogenous by varying the amount of yellow corn, soybean oil meal and animal fat. Adjustments in energy and protein content of diets were based on values of Titus (1955). Calcium and phosphorus adjustments were based on chemical analysis of ingredients, and the levels of these minerals determined in the mixed diets were in close agreement with the predicted values.

Each of the 10 experimental diets was fed to 3 pens of chicks in batteries and 2 pens on the floor in each trial. Experimental diets and tap water were given *ad libitum*.

Chicks were individually weighed and feed consumption determined at 4 weeks of age. Three males and 3 females from each floor pen and 2 males and 2 females from each battery pen were sacrificed at this time in trial 2 for bone ash determination.

Results and Discussion

Results from these trials (Tables 6 and 7) indicate that the requirement for calcium or phosphorus is similar for battery and floor grown chicks. No significant interactions were found between calcium or phosphorus and floor vs. battery grown chicks when measured by 28-day body weights or bone ash.

TABLE 6

Body weight and feed efficiency of chicks grown in batteries and floor pens when fed diets containing various levels of phosphorus and calcium (Trial 1)

Diet		4-week body weight (grams)							Feed/gain		
% P	% Ca	Males		Females		Both Sexes					
		B[1]	F[2]	B	F	B	F	Av	B	F	Av
0.48	0.60	428	406	370	380	399	393	396^b	1.58	1.71	1.65
	0.74	389	377	341	356	365	366	366^a	1.58	1.76	1.67
	0.88	379	374	342	341	360	358	359^a	1.58	1.70	1.64
0.59	0.60	508	507	429	450	469	478	474^{de}	1.53	1.64	1.59
	0.74	488	501	448	429	468	465	466^{cd}	1.52	1.63	1.58
	0.88	490	492	411	437	451	464	458^c	1.55	1.67	1.61
0.72	0.60	492	520	464	439	479	478	479^{de}	1.55	1.66	1.61
	0.74	509	520	414	451	462	485	474^{de}	1.54	1.65	1.60
	0.88	517	533	438	436	477	484	481^e	1.55	1.67	1.61
	1.10	516	530	444	437	480	483	482^e	1.52	1.62	1.57
Average		472	476	410	416	441	445	444	1.55	1.67	1.61

[1] B indicates battery grown chicks.

[2] F indicates floor grown chicks.

[3] Means having different superscripts are significantly different ($P = 0.05$).

TABLE 7

Body weight and bone ash of chicks grown in batteries and floor pens when fed diets containing various levels of phosphorus and calcium (Trial 2)

		4-week body weight (grams)							Bone ash (%)						
Diet		Males		Females		Both Sexes			Males		Females		Both Sexes		
% P	% Ca	B^1	F^2	B	F	B	F	Av^3	B	F	B	F	B	F	Av^3
0.48	0.60	417	394	398	361	407	378	393^c	35.6	32.6	36.3	34.9	35.9	33.7	34.8^b
	0.74	386	349	344	350	365	349	357^b	32.3	32.7	33.8	31.9	33.0	32.3	32.7^a
	0.88	348	352	319	314	333	333	333^a	32.7	31.1	33.2	31.9	33.0	31.5	32.3^a
0.59	0.60	445	466	409	410	427	438	433de	42.5	40.2	39.1	40.1	40.8	40.6	40.7^c
	0.74	469	473	426	422	448	447	447^{fg}	43.5	42.7	41.2	41.6	42.4	42.2	42.3^d
	0.88	479	472	421	395	450	433	442^{ef}	42.3	41.9	40.8	43.2	41.6	42.5	42.1^d
0.72	0.60	445	461	397	411	421	436	429^d	42.6	37.5	40.3	41.0	41.5	39.3	40.4^c
	0.74	526	461	451	409	489	435	462^g	44.1	41.2	42.8	41.8	43.5	41.5	42.5^d
	0.88	487	494	426	432	457	463	460^g	44.8	44.2	42.8	44.6	43.8	44.4	44.1^e
	1.10	494	473	422	419	458	446	452^{fg}	44.8	45.4	44.3	44.2	44.6	44.8	44.7^e
Average		450	439	401	392	426	416	421	40.5	39.0	39.5	39.5	40.0	39.3	39.7

[1] B indicates battery grown chicks.

[2] F indicates floor grown chicks.

[3] Means having different superscripts are significantly different.

These data, indicating a similar requirement for calcium or phosphorus by chicks grown either on wire or litter, are in direct contrast to data reported for the laying hen under comparable conditions (Crowley *et al.*, 1961; Harms *et al.*, 1961; Marr *et al.*, 1961; and Singsen *et al.*, 1961). It is rather difficult to attempt to explain the differential response between chicks and hens. Certainly these data would not substantiate the hypothesis that very much phosphorus is obtained from the feces. Although the phosphorus requirement determined for hens maintained on wire does not appear to be the same for hens in floor pens, this is apparently not the case for chicks.

The calcium X phosphorus interaction was highly significant when measured by body weight in both experiments and by bone ash in trial 2. This re-emphasizes the importance of the calcium-phosphorus ratio when the diet contains a sub-optimal level of either of the minerals, and agrees with recent data by Vandepopuliere *et al.* (1961), and Simco and Stephenson (1961).

Under conditions of these experiments 0.59 percent total phosphorus in the diet of females was just adequate to support maximum growth, but was not adequate for maximum bone ash (Tables 6 and 7). A significant increase in growth rate and bone ash was obtained with males when the total phosphorus content of the diet was increased from 0.59 to 0.72 percent, resulting in a significant interaction of sex X phosphorus. The interaction of sex and phosphorus has been previously reported (Vandepopuliere *et al.*, 1961) and re-emphasizes the importance of considering sex when determining phosphorus requirement of chicks.

A level of 0.60 percent calcium appeared to be adequate to support maximum growth in trial 1 (Table 6). However, males in trial 1 receiving higher levels made numerically but not significantly better growth. In trial 2 a significant improvement in body weight and bone ash was obtained when the level of calcium was increased from 0.60 to 0.72 percent (Table 7). Although there was a trend for the males to give a greater response than females to increased calcium, the interaction of calcium X sex was not statistically significant.

The calcium and phosphorus requirements of chicks as indicated by these data are slightly higher than those reported by Simco and Stephenson (1961). These workers used a different type of diet and a different source of calcium and phosphorus which may account for these differences in results.

CHAPTER 5

A COMPARISON OF PHOSPHORUS ASSAY TECHNIQUES WITH CHICKS

Studies have been conducted by many workers to establish the comparative utilization of phosphorus from various supplements by chicks. These reports are reviewed by Motzok et al. (1956) and Hurwitz (1964). In general these studies are based on relating percentage of tibia, beak, or toe ash of chicks fed a test phosphate to that of chicks fed a reference phosphate and establishing relative biological values.

Considerable variation in the assay procedure employed has existed. In certain assays constant calcium: phosphorus ratios were employed (Creech et al., 1956; Nelson and Peeler, 1961), while in others, constant calcium levels of 1 percent (Ammerman et al., 1960) or 1.2 percent (Gardiner et al., 1959) were used. Vandepopuliere et al. (1961) found that it was essential to consider both phosphorus level and Ca:P ratio when determining phosphorus availability. Nelson and Peeler (1964) enumerated problems involved in the development of a biological assay for feed phosphates. Among these was the question of holding the calcium level constant or having a constant calcium: phosphorus ratio in the assay diet.

Experiments were conducted to determine the influence of the calcium content of the assay diet and the method of interpretation of the data upon the relative availability of phosphorus from an inorganic phosphate.

Experimental Procedure

Feed grade dicalcium phosphate, generally considered to have a high phosphorus availability, was compared to soft phosphate, generally considered to be a less available source of phosphorus. Three regimens of calcium supplementation were compared which included: (1) a constant level of 1 percent Ca, (2) a constant Ca:P ratio of 2:1, and (3) a "sliding" Ca:P ratio. The "sliding" ratios consisted of a different Ca:P ratio for each level of phosphorus supplementation. Ratios were selected which were considered to permit optimum performance of the chick at each level of phosphorus supplementation.

Each of the phosphorus sources was added to the basal diet in amounts adequate to supply 0.05, 0.10, 0.20 and 0.30 percent supplemental phosphorus. These phosphorus levels were fed in combination with each of the 3 calcium regimens. The "sliding" Ca:P ratios used with the phosphorus levels were as follows:

% Added P	Total Ca: Total P
0.05	1.2:1
0.10	1.4:1
0.20	1.6:1
0.30	1.8:1

A simplified degerminated corn-soybean meal diet was used as the assay diet (Table 8). It was calculated to contain 22 percent protein and 1000 Calories of productive energy per pound. Analysis

TABLE 8

Composition of basal diet

Ingredients	Percent of diet
Degerminated corn meal	51.70
Cerelose	5.00
Soybean meal (50% protein)	34.00
Alfalfa meal (20% protein)	3.00
Iodized salt	0.40
Micro-ingredients[1]	0.90
Variable[2]	5.00
% Phosphorus	0.30
% Calcium	0.17

[1] Composition of micro-ingredients given in Table 5.

[2] Calcium and phosphorus levels were obtained by altering the levels of ground limestone, dicalcium phosphate, soft phosphate, and pulverized oat hulls.

indicated that the ingredients of the basal diet contributed 0.30 percent total phosphorus and 0.17 percent calcium. Desired calcium and phosphorus levels were attained by variation of the test phosphates and ground limestone. Pulverized oat hulls were used to maintain the diets isocaloric.

Each experimental diet was fed to 3 pens of 5 males and 5 female broiler chicks in 2 successive trials, giving a total of 50 chicks per treatment. The experimental diets and tap water were consumed ad libitum, beginning at one day of age.

At 28 days of age individual body weights were obtained. Two chicks of each sex from each pen were sacrificed and the right tibia removed for bone ash determination.

Results and Discussion

The type of calcium supplementation of the diet had a significant effect on growth and bone calcification (Table 9). The use of "sliding" Ca:P ratios resulted in significantly greater body weight and tibia ash of chicks fed diets supplemented with either dicalcium phosphate or soft phosphate, when compared to the use of 1 percent calcium or a constant 2:1 Ca:P ratio. The use of a constant 2:1 ratio promoted significantly greater weight gain and tibia ash for chicks fed diets supplemented with dicalcium phosphate than did the use of the constant 1 percent calcium. Use of the constant 2:1 Ca:P ratio supported significantly greater weight gains than did the use of 1

TABLE 9

Body weight and tibia ash of 28-day old chicks fed varying levels of phosphorus from two sources under three regimens of calcium supplementation

Calcium Regimen	Dietary % Ca	Total % P[1]	Body weight (gms)[1]		Tibia ash (%)[1]		Relative P Availability (%)[2]		
			DKP[3]	SP[4]	DKP	SP	A	B	C
1.00 %	1.00	0.35	291	249	28.9	29.8	. .	. .	. .
	1.00	0.40	337	266	32.2	29.8	. .	. .	. .
	1.00	0.50	399	322	40.8	33.8	. .	. .	. .
	1.00	0.60	405	378	42.2	39.0	. .	. .	. .
	Average		358^{c}	304^{a}	36.0^{b}	33.1^{a}	58.9	92.7	57.5
2:1 Ratio	0.70	0.35	340	300	30.5	31.0	. .	. .	. .
	0.80	0.40	377	306	34.8	32.9	. .	. .	. .
	1.00	0.50	407	337	39.9	34.5	. .	. .	. .
	1.20	0.60	415	355	43.1	37.5	. .	. .	. .
	Average		385^{d}	325^{b}	37.0^{c}	33.9^{a}	52.5	92.4	37.8
Sliding Ratio	0.42	0.35	378	320	33.9	34.0	. .	. .	. .
	0.56	0.40	389	326	36.5	34.3	. .	. .	. .
	0.80	0.50	422	371	41.5	36.4	. .	. .	. .
	1.08	0.60	420	381	43.0	39.3	. .	. .	. .
	Average		402^{e}	350^{c}	38.7^{d}	36.0^{b}	51.5	93.3	49.6

[1] Means having the same superscript do not differ significantly (P =0.05).

[2] Comparative biological availability of the phosphorus from soft phosphate based on tibia ash data. A= method of Nelson and Peeler (1961); B= method of Barauh *et al.* (1960); C= method of Combs (1955).

[3] DKP = feed grade dicalcium phosphate.

[4] SP = soft phosphate.

percent calcium in diets containing soft phosphate, but there was no difference in percent tibia ash.

The comparative availability of the phosphorus from soft phosphate as compared to the phosphorus from feed grade dicalcium phosphate was calculated using the tibia ash data in Table 9. Three methods of determining the comparative availability were used. The first method was that outlined by Nelson and Peeler (1961) in which the standard curve was a regression line obtained by plotting the percentage bone ash obtained at the various levels of added phosphorus against the logarithm of the added phosphorus. The calculated comparative availability of phosphorus from soft phosphate was 58.9, 52.5, and 51.5 percent with 1 percent Ca, 2:1 Ca:P ratio, and the "sliding" Ca:P ratio, respectively (Table 9). These values are greater than the 36 percent biological value reported by Nelson and Peeler (1961); however, the standard use in their test was beta-tricalcium phosphate which was of greater biological value than feed grade dicalcium phosphate.

The second method of determining comparative availability was that outlined by Barauh *et al.* (1960) in which the percentage tibia ash obtained with the test phosphate was divided by the percentage tibia ash obtained with the standard phosphate. Using this method, the comparative availability of the phosphorus from soft phosphate was calculated to be 92.7, 92.4, and 93.3 percent at the 1 percent calcium, 2:1 Ca:P ratio, and "sliding" ratio regimens, respectively (Table 9). However, the validity of this method is questioned by Nelson and Peeler (1961).

The third method used was that introduced by Combs (1955). In this procedure, the slope of the response line of the test phosphate (soft phosphate) was divided by the slope of the response line of the standard phosphate (feed grade dicalcium phosphate). Comparative availability values obtained by this method were 57.5, 37.8, and 49.6 percent for the 1 percent Ca, 2:1 Ca:P ratio, and "sliding" Ca:P ratio regimens, respectively (Table 9). With the exception of the value of 37.8 percent, these values compare favorably with those obtained using the method of Nelson and Peeler (1961).

More recently, Hurwitz (1964) proposed a procedure for the estimation of net phosphorus utilization on the basis of the phosphorus content of the tibia. This procedure offers another method of calculating biological values for feed grade phosphates.

It is apparent from the present experiment that variation in the procedure used to assay phosphate sources may greatly influence the results. The type of calcium supplementation or method of interpretation of the data may influence phosphorus utilization by the chick. Therefore, standardization of phosphorus assay technique appears to be desirable.

The use of different calcium levels or Ca:P ratios at different levels of phosphorus supplementation appears desirable in order to elicit maximum response of the chick and allow full utilization of the phosphorus. The variable Ca:P ratios selected for this study promoted greater body weight and bone calcification than did the use of a fixed

Ca level or fixed Ca:P ratio but tended to give a lower apparent biological value of the phosphorus from soft phosphorus.

CHAPTER 6

THE UTILIZATION OF VARIOUS SOURCES OF CALCIUM

Several sources of calcium are used in poultry feeding. Experimental data indicate that the biological availability of calcium from most of these sources is essentially equal. Buckner *et al.* (1923) reported that limestone or oyster shell serve equally well as sources of calcium for laying hens. The calcium in limestone, gypsum, calcium gluconate, and dolomite were reported by Deobald *et al.* (1936) to be equally available although differences in solubility of the supplements were observed. Buckner *et al.* (1929) compared calcium lactate, chloride, sulphate and carbonate and concluded that the calcium carbonate was most effective for egg production as judged by egg shell weight. Bethke *et al.* (1930) found no difference in the availability of calcium in the carbonate, sulfate, lactate, and phosphate salts or from limestone for bone formation in the growing chick on equal calcium intake. Dougherty and Gossman (1923) reported that limestone supported higher egg production than did oyster shell while Kennard (1925) found oyster shell to be superior to limestone. More recent reports indicate that calcium as calcium sulfate may be less available than calcium as calcium carbonate as measured by its inhibitory effect on antibiotic absorption (Donovan *et al.*, 1960).

Because of the effect of the dietary calcium:phosphorus ratio on growth and bone development, especially when diets low in phosphorus are fed, the availability of the calcium in the diet is of major importance. Two trials were conducted to determine the availability to chicks of calcium from sources available for commercial use.

Experimental Procedure

Trial 1.--Calcium sources tested were reagent calcium carbonate, reagent calcium sulfate, oyster shell, and ground limestone. The oyster shell was pulverized prior to its use.

Graded levels of the calcium from the various sources were added to a basal diet calculated to contain 20 percent protein and 1,000 kilocalories of productive energy (Table 10). This diet was calculated to contain 0.60 percent total phosphorus. Monosodium phosphate ($NaH_2PO_4 \cdot H_2O$) was used as the source of phosphorus. The basal diet was calculated to contain 0.17 percent calcium, all from plant sources.

Samples of the calcium sources were submitted to a laboratory for analysis. The calcium content as determined by this assay is shown in Table 11. Using these values in column 1, each calcium source was added to the basal diet in amounts sufficient to increase the total calcium content of the diet to 0.2, 0.3, 0.4, and 0.5 percent. This resulted in 16 experimental treatments.

Three groups of 5 male and 5 female day-old, broiler type chicks were assigned to each treatment. The chicks received the experimental diets and tap water ad libitum from day-old until 28 days of age. At this time individual body weights were obtained and 2 males

TABLE 10

Composition of basal diet

Ingredients	Percent of diet
Yellow corn	52.02
Soybean meal (50% protein)	31.25
Animal fat	1.53
Alfalfa meal (17% protein)	3.00
Monosodium acid phosphate	0.90
Iodized salt	0.40
Micro-ingredients[1]	0.90
Variable[2]	10.00

[1] Composition of micro-ingredients given in Table 5.

[2] Consists of a calcium supplement, cerelose, and corn oil.

TABLE 11

Analysis of calcium supplements

Supplement	Laboratory			
	A	B	C	D
		(% Calcium)		
$CaCO_3$ (USP reagent)	40.1	39.7	40.0	37.8
$CaSO_4$ (USP reagent)	23.3	23.3	23.3	. .
Ca Gluconate (USP reagent)	. .	. .	9.3	. .
Ground limestone "A"	36.3	37.8	37.7	38.4
Ground limestone "B"	. .	37.0	37.2	37.4
Ground oyster shell	39.2	38.4	37.5	35.0

and 2 females were sacrificed from each pen and the right tibia removed for bone ash determination.

Trial 2.--The second trial was a close replicate of the first with only minor changes. The lowest level of calcium tested in the first trial was 0.2 percent, of which only 0.03 percent came from the supplemental sources. Therefore, this level was eliminated from the design and levels of 0.3, 0.4, 0.5, 0.6 and 0.7 percent total calcium were fed. The same basal diet and same calcium supplements as used in the first experiment were used in the second experiment. In addition, calcium gluconate was used at the 0.4 and 0.6 percent levels, and another limestone source was used at all levels of calcium. This resulted in 27 experimental treatments. A similar number of chicks were assigned per treatment and brooded as indicated in trial 1.

Results and Discussion

Trial 1.--The results of this trial indicated that the calcium from the limestone supplement was more available than the calcium from reagent calcium carbonate or sulfate or ground oyster shell, as measured by bone ash (Table 12). However, there was no significant difference between the experimental groups as indicated by body weight at 28 days of age (Table 13). It is felt that the increased bone ash value was not meaningful since this was not repeated in the second trial. The analytical value used in this trial for limestone "A" may have been too low as indicated by analysis from other laboratories.

TABLE 12

Bone ash of chicks grown on diets with different calcium levels from various calcium supplements (Trial 1)

		Dietary calcium (%)				
Calcium Source	Sex	0.2	0.3	0.4	0.5	Av
				(% Bone ash)		
Reagent $CaCO_3$	M	26.7	29.8	33.3	35.6	31.5
	F	27.9	32.9	33.5	37.9	33.1
	Av	27.3	31.4	33.5	36.8	32.3
Reagent $CaSO_4$	M	27.4	29.5	33.4	35.5	31.4
	F	29.1	32.2	35.5	35.7	33.2
	Av	28.3	30.8	34.5	35.6	32.3
Oyster shell	M	26.8	30.1	33.7	36.3	31.7
	F	29.2	32.2	34.3	36.6	33.1
	Av	28.0	31.1	34.0	36.5	32.4
Limestone "A"	M	29.2	31.3	34.8	38.0	33.3
	F	28.4	32.6	36.4	37.5	33.7
	Av	28.8	31.9	35.6	37.8	35.5[1]
Average	M	27.5	30.2	33.8	36.4	
	F	28.7	32.5	34.9	36.9	
	Av	28.1	31.3	34.4	36.6	

[1] Differs significantly from other calcium sources (P = 0.05).

TABLE 13

Body weights of chicks grown on diets with different calcium levels from various calcium supplements (Trial 1)

Calcium Source	Sex	Dietary calcium (%)				
		0.2	0.3	0.4	0.5	Av
			Body weight (grams)			
Reagent $CaCO_3$	M	246	352	389	477	366
	F	214	315	357	401	324
	Av	230	334	373	444	345
Reagent $CaSO_4$	M	220	344	424	440	357
	F	222	334	390	390	334
	Av	221	339	407	415	346
Oyster shell	M	221	356	420	488	371
	F	199	340	378	440	339
	Av	210	348	399	464	355
Limestone "A"	M	254	354	434	472	378
	F	193	353	399	395	335
	Av	224	353	416	434	357
Average	M	235	352	417	469	
	F	207	336	381	409	
	Av	221	344	399	439	

Increasing the calcium level of the diet significantly improved body weight and increased the percent tibia ash of chicks (Tables 12 and 13). Male chickens were significantly heavier than female chicks, but the females had a higher average percent tibia ash.

There was no calcium source X level interaction observed for either body weight or percent bone ash, indicating that each of the supplements had essentially the same availability at all calcium levels tested.

Trial 2.--No significant differences were observed between any of the calcium supplements tested as measured by both body weight and tibia ash (Tables 14 and 15).

Analysis of the data indicated that a level of 0.50 percent total calcium was sufficient to support maximum body weight (Table 14), but that 0.60 percent total calcium was required for maximum bone calcification (Table 15) under the conditions of this experiment.

The body weight of males was significantly higher than that of females (Table 14). This effect was not altered by the calcium level of the diet. Females tended to have a higher percent tibia ash than did males (Table 15); however, this effect was significantly altered by the calcium level of the diet. Females were able to tolerate lower levels of calcium than were males as indicated by percent tibia ash.

TABLE 14

Body weight of chicks fed diets with different calcium levels from various calcium supplements (Trial 2)

Calcium	Sex	Dietary calcium (%)					Average[1,2]
		0.30	0.40	0.50	0.60	0.70	
		Body weight (grams)[3]					
Reagent $CaCO_3$	M	376	430	467	476	464	443
	F	372	409	415	404	416	403
	Av	374^{d}	420^{ef}	440^{fg}	440^{fg}	440^{fg}	423
Reagent $CaSO_4$	M	378	444	464	460	456	440
	F	346	399	410	410	398	393
	Av	362^{d}	421^{ef}	437^{fg}	436^{fg}	427^{efg}	417
Oyster Shell	M	362	458	472	486	468	449
	F	357	404	415	416	410	400
	Av	360^{d}	431^{efg}	444^{g}	448^{g}	439^{fg}	424
Limestone "A"	M	401	436	478	449	453	443
	F	361	383	417	408	401	394
	Av	381^{d}	410^{e}	448^{g}	428^{efg}	427^{efg}	419
Limestone "B"	M	392	442	455	458	434	436
	F	368	401	415	396	399	396
	Av	380^{d}	421^{ef}	435^{fg}	427^{efg}	428^{efg}	418
Calcium Gluconate	M	. .	448	. .	465	. .	. .
	F	. .	402	. .	399	. .	. .
	Av	. .	425^{efg}	. .	432^{efg}	. .	. .
Average[1]	M	382	442	460	466	455	442
	F	361	399	413	407	405	397
	Av	372^{a}	420^{b}	437^{c}	437^{c}	430^{c}	420

[1] Does not include calcium gluconate groups.

[2] Means do not differ significantly ($P=0.05$).

[3] Means with different superscripts are significantly different according to Duncan's multiple range test (1955).

TABLE 15

Tibia ash of chicks fed diets with different calcium levels from various calcium supplements (Trial 2)

Calcium	Sex	Dietary calcium (%)					Average[1,2]
		0.3	0.4	0.5	0.6	0.7	
		Tibia ash (%)[3]					
Reagent $CaCO_3$	M	32.0	35.3	37.1	38.4	37.8	36.1
	F	35.4	37.6	38.5	39.5	39.0	38.0
	Av	33.7^{e}	36.5^{f}	37.8^{fgh}	38.9^{ij}	38.4^{hij}	37.1
Reagent $CaSO_4$	M	32.5	36.2	37.8	38.2	37.3	36.4
	F	33.9	36.9	38.7	38.6	39.6	37.5
	Av	33.2^{e}	36.5^{f}	38.2^{hi}	38.4^{hij}	38.4^{hij}	36.9
Oyster Shell	M	32.7	35.5	37.7	39.4	39.2	36.9
	F	33.7	37.1	39.6	38.7	40.7	37.9
	Av	33.2^{e}	36.3^{f}	38.7^{hij}	39.0^{ij}	39.9^{j}	37.4
Limestone "A"	M	33.3	35.6	37.2	38.7	39.2	36.8
	F	34.1	37.2	37.5	39.3	38.8	37.4
	Av	33.7	36.4^{f}	37.3^{fgh}	38.9^{ij}	38.9^{ij}	37.0
Limestone "B"	M	33.3	36.6	37.6	38.5	38.8	36.9
	F	34.9	36.6	38.3	38.6	38.7	37.4
	Av	34.1^{e}	36.6^{fg}	37.9^{ghi}	38.5^{hij}	38.8^{ij}	37.2
Calcium Gluconate	M	. .	35.8	. .	37.3	. .	. .
	F	. .	37.9	. .	40.2	. .	. .
	Av	. .	36.9^{fg}	. .	38.8^{ij}	. .	. .
Average[1]	M	32.8	35.8	37.5	38.6	38.5	36.6
	F	34.4	37.1	38.5	38.9	39.4	37.6
	Av	33.6^{a}	36.5^{b}	38.0^{c}	38.8^{d}	38.9^{d}	37.1

[1] Does not include calcium gluconate groups.

[2] Means do not differ significantly ($P=0.05$).

[3] Means with different superscripts are significantly different according to Duncan's multiple range test (1955).

CHAPTER 7

THE EFFECT OF VITAMIN D ON PHOSPHORUS UTILIZATION

Within the past few years much attention has been directed toward a re-evaluation of the phosphorus requirements of chicks (Simco and Stephenson, 1961; Lillie *et al.*, 1961; Formica *et al.*, 1961; Vandepopuliere *et al.*, 1961). However, varying results have been reported. A part of the variability in the results obtained may have been due to the inter-relationship in the diet of calcium, phosphorus, and vitamin D, which has long been known to be important for satisfactory growth and bone development (Bethke *et al.*, 1928; Hart *et al.*, 1930; Wilgus, 1931; McChesney and Giacomino, 1945; Carver *et al.*, 1946; Migicovsky and Emslie, 1947). To gain additional information about the inter-relationship of these factors, experiments were conducted to determine the effects of various calcium:phosphorus ratios and levels of vitamin D_3 on the utilization of phosphorus by broiler-type chicks.

The Interaction of Calcium, Phosphorus, and Vitamin D

Experimental Procedure

Two trials were conducted to evaluate the degree of interaction existing between the vitamin D, calcium and phosphorus levels in the diet of chicks. The design of the 2 trials involved a 3X3X3 factorial arrangement of treatments, with 3 calcium:phosphorus ratios (1:1, 1.4:1, and 1.8:1), 3 levels of phosphorus (0.48, 0.59, and 0.70 percent total phosphorus), and 3 levels of vitamin D_3. The vitamin D_3 levels were altered for the 2 trials, with 45, 90, and 180 I.C.U./lb. of diet being used in the first and 90, 180, and 360 I.C.U./lb. being used in the second.

The composition of the basal diet used in this experiment is shown in Table 16. By analysis, the basal diet supplied 0.39 percent phosphorus, all as organic phosphorus. Reagent grade calcium carbonate and monosodium phosphate ($NaH_2PO_4 \cdot H_2O$) were used to supply the desired levels of calcium and phosphorus. A commercial source of vitamin D_3 (200,000 USP units per gram guaranteed) was obtained and the potency determined by the average of 3 independent laboratory reports using different analytical techniques (233,000 I.C.U. per gram). Cerelose and corn oil were used to keep the diets iso-caloric. Samples of the diet were assayed for calcium and phosphorus, and the results were in close agreement with calculated values. The basal diet for all treatments, with the exception of the variable ingredients, was mixed in a single batch, and aliquot parts were used for each experimental diet. The diets were mixed the day before the trial began and stored at 55° F. until fed.

Day-old broiler chicks were randomly assigned into groups of 5 male and 5 female chicks, and placed in battery brooders. Three groups were assigned to each treatment. The experimental diets and tap water were given ad libitum. All sources of ultra-violet light were eliminated from the battery room.

TABLE 16

Composition of basal diet

Ingredient	Percent of diet
Yellow corn	50.0
Soybean meal (50% protein)	34.0
Animal fat	1.7
Alfalfa meal (17% protein)	3.0
Iodized salt	0.4
Micro-ingredients[1]	0.9
Variables[2]	10.0

[1] Composition of micro-ingredients given in Table 5 with removal of vitamin D.

[2] Variable ingredients included reagent $CaCO_3$, $NaH_2PO_4 \cdot H_2O$ and corn oil.

At 28 days of age the chicks were individually weighed, and the experiments terminated. Feed consumption per group was determined, and feed conversion was calculated. Two males and 2 females from each group were sacrificed for bone ash determination.

Results and Discussion

Increasing the level of phosphorus in the diet from 0.48 to 0.70 percent resulted in a highly significant increase in average body weight (Table 17) and percent bone ash (Table 18). These effects were best fitted by quadratic equations, indicating that the optimum phosphorus level had been reached.

Widening the calcium:phosphorus ratio of the diet from 1:1 to 1.4:1 significantly increased body weight and percent bone ash (Tables 17 and 18). A further increase to a 1.8:1 ratio did not affect body weight but lowered the percent bone ash; however, this reduction was not statistically significant.

A highly significant increase in body weight and bone ash was observed when the vitamin D_3 level of the diet increased from 45 I.C.U. to 180 I.C.U. per pound (Tables 17 and 18). This increase was linear, indicating that higher levels of vitamin D_3 were beneficial, and promoted the change in levels for the second trial.

Several interactions between the factors were observed. The interaction of Ca:P ration X phosphorus level of the diet was highly significant for both body weight and bone ash (Tables 17 and 18). Vitamin D_3 levels and Ca:P ratios also interacted in a significant

TABLE 17

Body weight of chicks grown on diets varying in levels of calcium, phosphorus, and vitamin D_3

		% Phosphorus			
Ca:P Ratio	Vit. D I.C.U./lb.	0.48	0.59	0.70	Average
1:1	45	204	249	260	238
	90	256	299	349	295
	180	360	384	416	387
	360	383	420	424	409
	Av	300	338	362	332
1.4:1	45	256	329	374	319
	90	314	396	395	368
	180	328	395	416	379
	360	355	408	392	385
	Av	313	382	394	363
1.8:1	45	259	363	399	340
	90	291	376	405	357
	180	311	389	417	372
	360	314	367	396	359
	Av	294	374	404	357
Average	45	239	314	344	299
	90	287	357	383	340
	180	333	398	416	379
	360	350	398	404	384
	Av	302	365	387	351

TABLE 18

Percent bone ash of chicks grown on diets varying in levels of calcium, phosphorus, and vitamin D_3

Ca:P Ratio	Vit. D. I.C.U./lb.	% Phosphorus			
		0.48	0.59	0.70	Average
1:1	45	29.0	28.8	31.3	29.7
	90	29.4	32.3	34.9	32.2
	180	33.9	37.5	39.6	37.0
	360	37.3	39.5	40.2	39.0
	Av	32.4	34.6	36.5	34.5
1.4:1	45	27.4	32.1	37.0	32.2
	90	29.0	37.3	39.6	35.3
	180	31.4	39.0	42.0	37.5
	360	33.0	40.5	43.2	38.9
	Av	30.2	37.2	40.5	35.9
1.8:1	45	26.7	34.5	39.2	33.5
	90	26.9	36.7	40.3	34.6
	180	28.4	38.4	42.6	36.5
	360	29.4	38.3	40.8	36.2
	Av	27.8	37.0	40.7	35.2
Average	45	27.7	31.8	35.8	31.8
	90	28.4	35.4	38.3	34.1
	180	31.2	38.4	41.4	37.0
	360	33.4	39.5	41.4	38.1
	Av	30.2	36.3	39.2	35.3

manner for both of these criteria. The interaction of phosphorus levels and vitamin D_3 levels of the diet was significant for body weights but not for percent bone ash (Tables 17 and 18). No interaction of the three major factors, Ca:P ratio, vitamin D_3 and phosphorus levels was observed for either body weight or bone ash.

Males had greater body weights than did females, but in general the females had a higher percent bone ash. Lower requirements for mineralization by females were indicated by sex X Ca:P ratio and sex X vitamin D_3 level interactions.

The second trial was almost identical with the first, having the same phosphorus levels and Ca:P ratios but increased vitamin D_3 levels. Therefore, similar results were expected. Both body weight and percent bone ash were significantly increased when the phosphorus level of the diet was increased from 0.48 to 0.70 percent (Tables 17 and 18). These effects were best described as fitting a quadratic equation, indicating that an optimum level was reached.

A calcium:phosphorus ratio of 1.4:1 did not increase body weights when compared to the 1:1 ratio, but increased the percent bone ash (Tables 17 and 18). A further spread of the Ca:P ratio to 1.8:1 lowered both body weight and bone ash.

Increased levels of vitamin D_3 resulted in increased body weight and percent bone ash (Tables 17 and 18). The response of body weight was best described as a quadratic effect, while percent bone ash assumed a linear effect.

The interaction of Ca:P ratio X phosphorus level of the diet remained highly significant for both body weight and percent bone ash (Tables 17 and 18). Ca:P ratios and vitamin D_3 levels also interacted in a highly significant manner for these criteria. A significant interaction of phosphorus X vitamin D_3 level was observed for both criteria. A significant phosphorus X Ca:P ratio X vitamin D_3 interaction occurred with body weight, but not with percent bone ash (Tables 17 and 18).

As in the first trial, females weighed less but in general had a greater percent bone ash.

Analysis of the data showed that the treatment groups common to both trials 1 and 2 (those diets containing 90 and 180 I.C.U./lb. of vitamin D_3) were true replicates. Therefore, the results of the 2 trials were combined for discussion.

The response to increasing the phosphorus level of the diet from 0.48 to 0.70 percent indicated that the optimum phosphorus level for both body weight and bone ash was between 0.59 and 0.70 percent of the diet (Figure 1). This appeared to be true for both sexes, since there was no sex X phosphorus interaction.

Interpolation of the data in Figure 1 indicates that the phosphorus requirement is approximately 0.64 percent. This agrees closely with recent work using similar basal diets and experimental conditions (Vandepopuliere et al., 1961). The response in body weight closely paralleled that of bone ash.

Calcium:phosphorus ratios of 1:1 significantly depressed both body weight and percent of bone ash when compared to the 1.4:1 ratio,

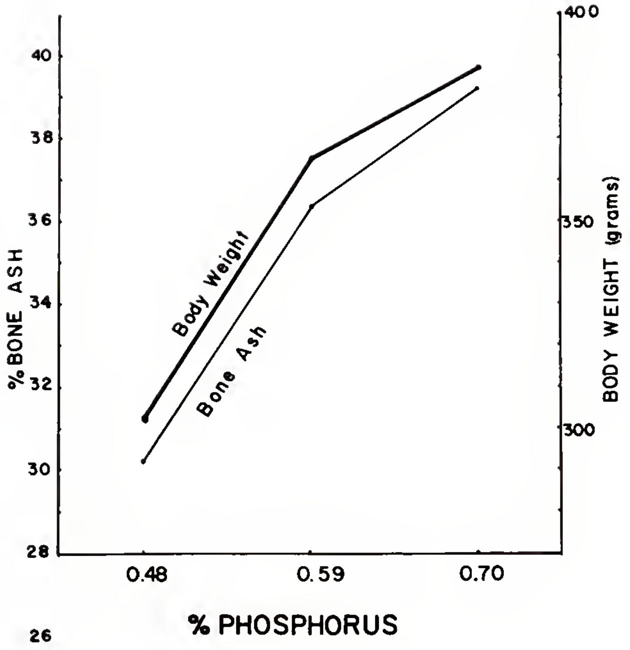

FIGURE 1

Bone ash and body weight of chicks receiving various dietary phosphorus levels over all levels of phosphorus and vitamin D_3 (Figure 2).

The response to increased levels of vitamin D_3 in these tests indicated that 180 I.C.U./lb. was adequate for maximum body weight but that 360 I.C.U./lb. further increased the percent bone ash (Figure 3).

Tibia ash appeared to be a more sensitive criterion than body weight for studying the adequacy of the calcium, phosphorus, and vitamin D_3 levels of the diet. Therefore, this measurement is used to illustrate the interaction that occurred in these experiments.

The interaction of Ca:P ratios and phosphorus levels is illustrated in Figure 4. At 0.48 percent phosphorus, a sub-optimal level, Ca:P ratios of 1.4:1 and 1.8:1 significantly depressed the percent bone ash, as compared to the 1:1 ratio. As the level of phosphorus more closely approached the requirement, between 0.59 and 0.70 percent, the wider ratios resulted in increased tibia ash, indicating a greater tolerance and in fact a necessity for increased ratios as the level of phosphorus increases.

A primary function of vitamin D has been shown to be the enhancement of calcium absorption of the intestinal tract (Wasserman et al., 1957; Greenberg, 1945). Figure 5 illustrates this effect and indicates that the response to the increased vitamin D_3 levels decreased as the Ca:P ratio widened. At the lower Ca:P ratios the calcium level was no doubt sub-optimal, even though the phosphorus level may have been adequate. As the ratio widened, more calcium was present, and the response to the increased levels of vitamin D_3 was less.

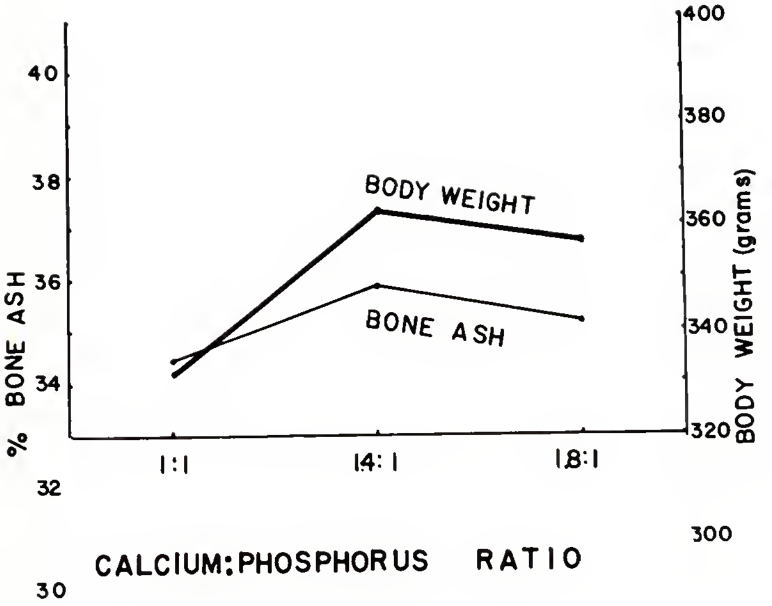

FIGURE 2

Bone ash and body weight of chicks receiving various calcium:phosphorus ratios

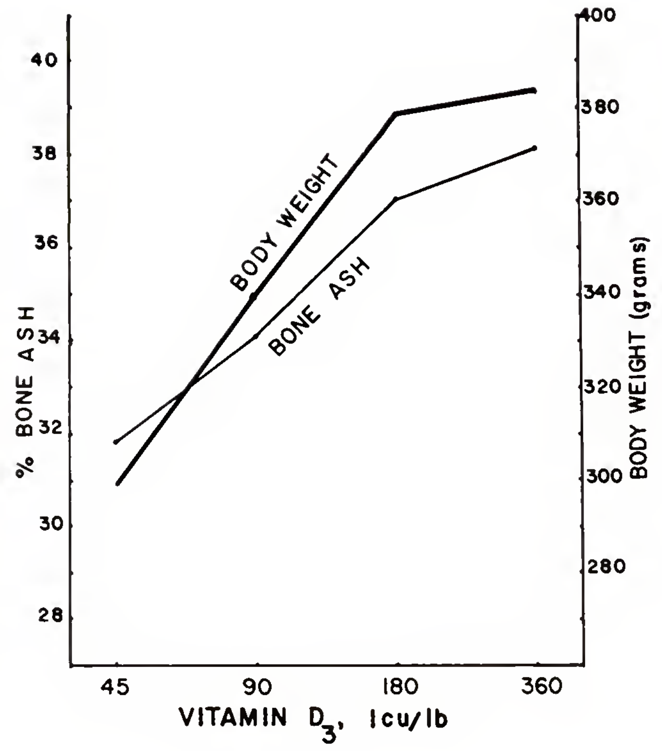

FIGURE 3

Bone ash and body weight of chicks receiving various dietary levels of vitamin D_3

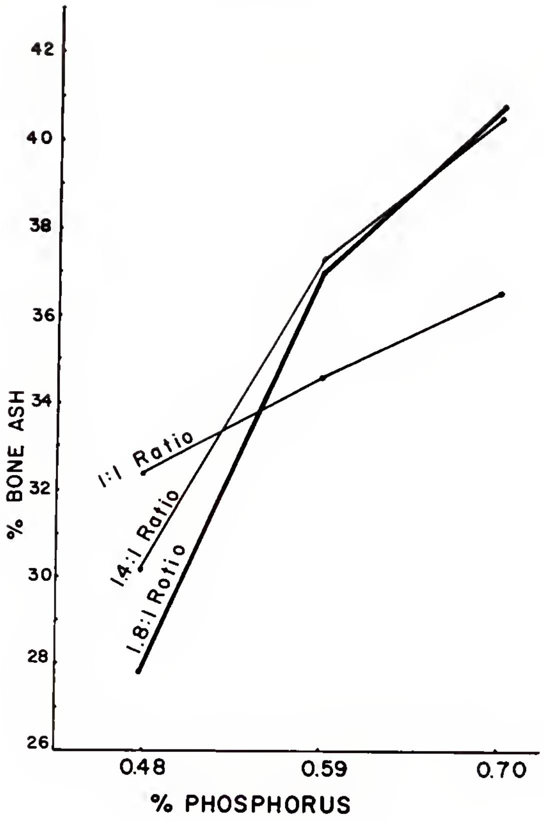

FIGURE 4

Bone ash of chicks receiving various Ca:P ratios
with various phosphorus levels

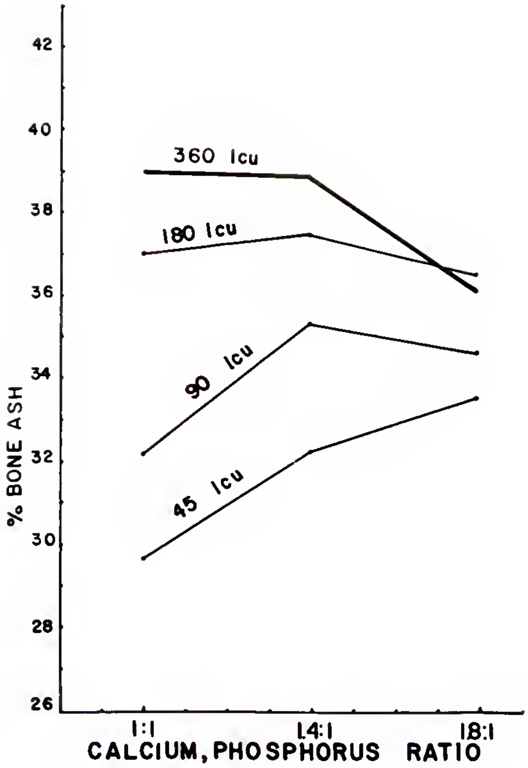

FIGURE 5

Bone ash of chicks receiving various levels of vitamin D_3 with various calcium:phosphorus ratios

Although studies by Cohn and Greenberg (1939) indicated that vitamin D did not enhance phosphate absorption in rats reared on a vitamin D deficient, high calcium--low phosphorus diet, an interaction of vitamin D_3 and phosphorus level was noted in this experiment. Figure 6 indicates that as the level of phosphorus more closely approached the requirement, between 0.59 and 0.70 percent of the diet, the response to the increased vitamin D_3 deviated from a linear to a quadratic effect. Rather than being an effect of the vitamin D_3 on phosphorus utilization per se (an actual vitamin D_3 X phosphorus interaction), this response may merely reflect a vitamin D_3 effect on the increased calcium levels occurring as the phosphorus level increased, since the calcium is present as a ratio of the phosphorus and not as a constant level. This graph also indicates that at sub-optimal levels of vitamin D the phosphorus requirement may be in excess of 0.70 percent, as indicated by the linear response of the 45 I.C.U./lb. level of vitamin D_3.

No significant interaction of Ca:P ratios and vitamin D_3 and phosphorus levels was observed when the data were combined from the 2 experiments. This was true for both bone ash and body weights. However, certain trends were evident, and definite observations can be made concerning the over-all interaction of this triumvirate. Widening the calcium:phosphorus ratios at sub-optimal levels of phosphorus resulted in decreasing the body weight or percent bone ash. Chicks tolerated the wider ratios more effectively with increased levels of vitamin D_3, up to 360 I.C.U./lb. in these tests.

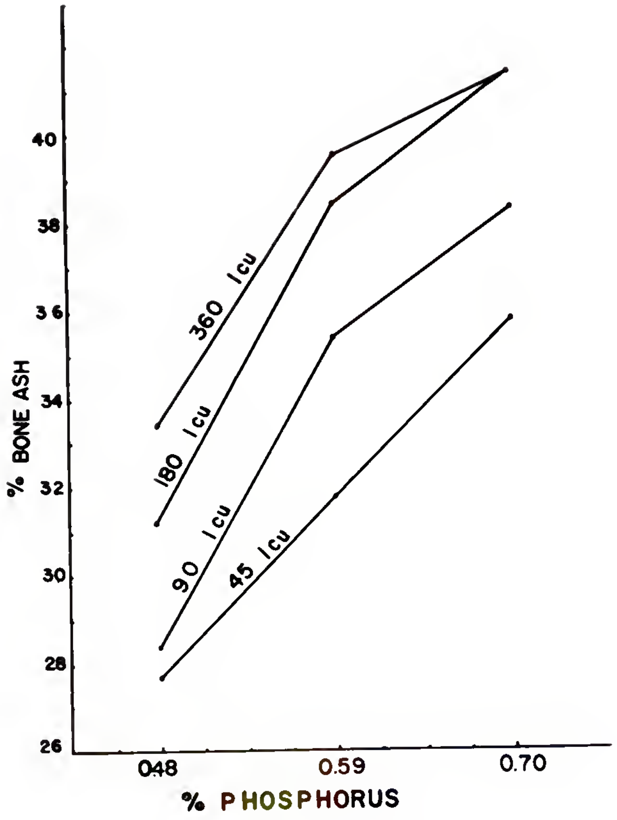

FIGURE 6

Bone ash of chicks receiving various levels of vitamin D_3 with various phosphorus levels

As the level of phosphorus increased, wider calcium:phosphorus ratios were tolerated or in fact desirable. This may be due not only to the actual calcium:phosphorus ratio but also to the total amount of calcium and phosphorus present. The response to increased vitamin D_3 became less as calcium and phosphorus levels approached the optimum.

The Vitamin D Requirement of the Chick as Influenced by the Dietary Calcium and Phosphorus Level

Results of the previous experiment indicated that dietary vitamin D_3 levels of 360 I.C.U. per pound increased body weight and percent bone ash of chicks. However, the response to higher levels of this vitamin became less as calcium and phosphorus levels approached optimum. To gain additional information regarding the effect of increased vitamin D_3 levels and the relationship of this vitamin to the calcium and phosphorus content of the diet, feeding trials were conducted under conditions of practical-type rearing as well as in battery brooders isolated from sources of ultraviolet light.

Experimental Procedure

Battery brooder studies.--A factorial arrangement with 2 calcium levels (0.50 and 1.00 percent), 2 phosphorus levels (0.50 and 0.70 percent total phosphorus) and 7 levels of vitamin D_3 was used. Levels of vitamin D_3 compared were 45, 90, 180, 360, 720, 3600 and 7200 I.C.U. per pound of feed. This resulted in diets which were (1) adequate in both calcium and phosphorus, (2) adequate in calcium with sub-optimal phosphorus, (3) sub-optimal calcium with adequate phosphorus, and (4) sub-optimal in both calcium and phosphorus.

Three pens of 5 male and 5 female day-old broiler chicks were randomly assigned to each dietary treatment in 2 successive trials, giving a total of 60 chicks per treatment, placed in battery brooders. All sources of ultraviolet light were eliminated from the experimental room.

The composition of the basal diet is given in Table 19. Desired levels of calcium and phosphorus were attained by a variation in the level of reagent grade calcium carbonate and reagent grade monosodium phosphate ($NaH_2PO_4 \cdot H_2O$). Sand was used as a non-nutritive filler to keep energy and protein levels constant. The vitamin D_3 supplement was described in the previous experiment. The diets were mixed 2 days prior to the beginning of each experiment and stored at 55° F. until fed. The experimental diets and tap water were fed *ad libitum*. At 28 days of age individual body weights were obtained. Two males and 2 female chicks from each pen were sacrificed for bone ash determination.

Floor pen studies.--To study the vitamin D_3 requirement of the broiler chick under conditions usually encountered in commercial flocks, a trial was conducted in floor pens in a broiler house in which no attempt was made to exclude sources of natural light. The house contained 4 rows of pens, 2 of which were along the outer wall with 2 inner rows. Four replicate pens of 10 male and 10 female chicks were assigned to each dietary treatment with the restriction that a pen from each of the 4 rows be used. The pens were 5 x 5 feet in size.

TABLE 19

Composition of diets

Ingredients	Battery	Floor		
		1	2	3
		Percent of diet		
Yellow corn meal	51.70	56.08	56.08	56.08
Soybean meal (50% protein)	34.00	34.00	34.00	34.00
Dehydrated alfalfa meal (20% protein)	3.00	3.00	3.00	3.00
Ground limestone	. .	0.22	0.75	1.27
Dicalcium phosphate	. .	1.35	1.35	1.35
Animal fat	. .	3.00	3.00	3.00
Iodized salt	0.40	0.40	0.40	0.40
Micro-ingredients[1]	0.90	0.90	0.90	0.90
Variable[2]	10.00	. .	. .	. .
Sand	. .	1.05	0.52	. .
% Protein	22.4	22.6	22.6	22.6
Productive energy (Cal./lb.)	928	981	981	981
% Calcium[2]	. .	0.60	0.80	1.00
% Phosphorus[2]	. .	0.65	0.65	0.65

[1] As outlined in Table 5 with removal of vitamin D.

[2] Calcium and phosphorus levels were adjusted by altering the level of variable ingredients consisting of calcium carbonate, monosodium phosphate, yellow corn and sand.

A 3 X 6 factorial arrangement of treatments was used. This consisted of calcium levels of 0.60, 0.80, and 1.00 percent with a vitamin D_3 level of 45, 90, 180, 360, 720, and 3600 I.C.U. per pound. All diets contained 0.65 percent total phosphorus and were iso-caloric and iso-nitrogenous. The composition of the diets containing the three calcium levels is shown in Table 19. The vitamin D_3 supplement used in the battery brooder trials was used to supply the desired levels of this vitamin.

Body weight and feed consumption were determined at 8 weeks of age. Two male and 2 female chicks from each pen were sacrificied for determination of bone ash.

Results and Discussion

Battery brooder studies.--The vitamin D_3 requirement of the chicks used in these studies was highly dependent upon the calcium and phosphorus level of the diet. With levels of 1.00 percent calcium and 0.70 percent phosphorus, maximum body weight and bone ash were obtained with 90 I.C.U. of vitamin D_3 per pound (Tables 20 and 21). This is in agreement with the vitamin D_3 requirement as suggested by the National Research Council (1960). When levels of calcium or phosphorus were sub-optimal, the need for higher vitamin D_3 levels was observed. With a dietary calcium level of 1.00 percent and a total phosphorus level of 0.50 percent, 3600 I.C.U. of vitamin D_3 per pound was needed for optimum body weight and bone ash. At a dietary calcium level of 0.50 percent, 720 I.C.U. of vitamin D_3 per pound was required for maximum body weight and bone ash at both phosphorus levels.

TABLE 20

Body weight of chicks fed different levels of vitamin D_3 at different calcium and phosphorus levels (battery brooder studies)

% Ca	% P	Vitamin D_3 (I.C.U./lb.)							
		45	90	180	360	720	3600	7200	Average
		Body Weight (grams)[1]							
1.00	0.70	438^{gh}	481^{jk}	501^{k}	479^{jk}	489^{jk}	473^{ij}	489^{jk}	479
	0.50	310^{d}	334^{de}	353^{ef}	366^{f}	412^{g}	433^{gh}	424^{g}	376
0.50	0.70	209^{a}	233^{b}	327^{d}	467^{ij}	487^{jk}	476^{ijk}	452^{hi}	377
	0.50	200^{a}	279^{c}	369^{f}	420^{g}	468^{ij}	489^{jk}	477^{ijk}	386
Average		289	332	385	433	464	468	460	

[1] Means are average weights of 30 male and 30 female chicks. Means bearing the same superscripts do not differ significantly ($P = 0.05$).

TABLE 21

Tibia ash of chicks fed different levels of vitamin D_3 at different calcium and phosphorus levels (battery brooder studies)

% Ca	% P	Vitamin D_3 (I.C.U./lb.)							
		45	90	180	360	720	3600	7200	Average
		(% Tibia Ash)[1]							
1.00	0.70	41.1^{b}	42.4^{a}	42.5^{a}	42.9^{a}	42.3^{a}	43.2^{a}	42.9^{a}	42.4
	0.50	31.3^{k}	31.3^{k}	32.5^{k}	33.9^{i}	36.2^{h}	37.3^{fg}	36.7^{g}	34.2
0.50	0.70	33.7^{i}	33.8^{i}	36.8^{gh}	37.3^{ef}	38.1^{de}	37.6^{de}	38.5^{cd}	36.5
	0.50	32.7^{k}	33.5^{i}	35.7^{h}	36.5^{fgh}	37.8^{de}	38.7^{cd}	39.1^{c}	36.3
Average		34.7	35.2	36.8	37.7	38.6	39.2	39.3	

[1] Expressed as percent fat-free, moisture-free bone. Means are average of 12 male and 12 female chicks. Means bearing same superscripts do not differ significantly ($P = 0.05$).

Floor pen studies.--The 56-day body weight and tibia ash of the chicks were significantly affected by the dietary calcium and vitamin D_3 levels (Tables 22 and 23). There was a significant interaction observed between the dietary calcium level and the vitamin D_3 level. At 0.60 percent calcium, 360 I.C.U. of vitamin D_3 per pound gave significantly maximum body weight and tibia ash. Increased levels of this vitamin numerically increased body weight but significantly reduced the tibia ash. When the dietary calcium level was increased to 0.80 percent a level of 90 I.C.U. of vitamin D_3 per pound gave significantly maximum body weight and tibia ash. Higher levels of vitamin D_3 numerically increased the body weight but had no influence on tibia ash. At a dietary calcium level of 1.00 percent, the vitamin D_3 response was altered. Increasing the level of vitamin D_3 up to 180 I.C.U. per pound numerically increased body weight but further increases in the level of this vitamin tended to reduce growth slightly. None of the groups, however, differed significantly in body weight or in tibia ash.

Feed utilization, expressed as grams of feed consumed per gram of gain, was closely related to body weight. In general, an increased body weight was associated with a decrease in the feed required to produce a unit of gain (Table 24).

The results of these studies confirm the close interrelationship of calcium, phosphorus, and vitamin D_3 in the diet of chicks. This relationship has been observed by many authors, yet few have attempted to establish a requirement for this vitamin in the diet of rapidly growing chicks.

TABLE 22

Body weight of chicks fed different levels of vitamin D_3 at different dietary calcium levels (floor pen study)

Vitamin D_3 (I.C.U./lb.)	% Calcium 0.60	0.80	1.00	Average
	Body Weight (grams)[1]			
45	671^{a}	1143^{c}	1235^{d}	1017
90	967^{b}	1276^{def}	1256^{def}	1167
180	1168^{c}	1290^{def}	1280^{def}	1247
360	1246^{d}	1291^{def}	1277^{def}	1271
720	1264^{def}	1308^{ef}	1257^{de}	1276
3600	1292^{def}	1321^{f}	1268^{def}	1294
Average	1101	1272	1263	

[1] Means are average weights of 40 male and 40 female chicks. Means bearing the same superscript do not differ significantly ($P = 0.05$).

TABLE 23

Tibia ash of chicks fed different levels of vitamin D_3 at different dietary calcium levels (floor pen study)

Vitamin D_3 (I.C.U./lb.)	% Calcium			
	0.60	0.80	1.00	Average
	% Tibia Ash[1]			
45	42.0^{a}	44.9^{bcd}	45.1^{cde}	43.9
90	43.1^{b}	45.8^{de}	46.4^{e}	45.1
180	43.8^{bc}	46.2^{e}	46.7^{e}	45.6
360	46.2^{e}	45.6^{cde}	45.9^{de}	45.9
720	44.9^{bcde}	45.9^{de}	46.4^{e}	45.8
3600	44.2^{bcd}	45.9^{de}	46.8^{e}	45.7
Average	44.0	45.7	46.2	

[1] Expressed as percent of fat-free, moisture-free bone. Means are average of 8 male and 8 female chicks. Means having the same superscript do not differ significantly ($P = 0.05$).

TABLE 24

Feed utilization of chicks fed different levels of vitamin D_3 at different dietary calcium levels (floor pen study)

Vitamin D_3 (I.C.U./lb.)	% Calcium 0.60	0.80	1.00	Average
		(grams feed/gram gain)[1]		
45	2.52^{e}	2.36^{d}	2.25^{abc}	2.38
90	2.37^{d}	2.28^{bc}	2.23^{abc}	2.29
180	2.30^{cd}	2.23^{abc}	2.23^{abc}	2.25
360	2.26^{bc}	2.26^{bc}	2.29^{bcd}	2.27
720	2.26^{bc}	2.25^{abc}	2.22^{ab}	2.24
3600	2.26^{bc}	2.18^{a}	2.25^{abc}	2.23
Average	2.33	2.26	2.25	

[1] Means are average of four pens, each containing 10 male and 10 female chicks. Means having the same superscript do not differ significantly (P =0.05).

Under the conditions of the experiment reported herein, 90 I. C. U. of vitamin D_3 per pound as suggested by the National Research Countil is adequate to support maximum growth and bone ash at the calcium and phosphorus levels recommended by this group (1.0 percent Ca and 0.6 percent P). Higher levels did not significantly influence body weight or bone ash at 4 or 8 weeks of age.

In recent years, however, the calcium requirement of the chicks has undergone considerable study with some disagreement as to the optimum level required. These reports have been recently reviewed by Waldroup et al., (1963). With the possible advent of lowered calcium levels in the diet, the need to re-examine the vitamin D_3 requirement becomes apparent from the results of the present studies. Lowering the calcium level to 0.8, 0.6 or 0.5 percent significantly altered the vitamin D_3 requirement. This was true for both age groups and regardless of whether the chicks were grown in battery brooders in the absence of ultraviolet light or in floor pens in a conventional broiler house. Since a primary function of vitamin D has been shown to be the enhancement of calcium absorption from the intestial tract (Greenberg, 1945; Wasserman et al., 1957) it is logical to assume that the response to vitamin D supplementation would be increased as the calcium level of the diet is lowered.

The relationship of vitamin D and phosphorus is less well defined. The results of these studies indicated an interaction of vitamin D and phosphorus; however, this may be a reflection of the calcium:phosphorus interaction rather than a true effect of the vitamin on phosphorus utilization. This hypothesis is strengthened by the early report by Cohn and Greenberg (1939) which indicated that vitamin D did not enhance phosphate absorption in rats reared on a vitamin D deficient, high calcium-low phosphorus diet. The importance of adequate vitamin D level in a biological assay for either calcium or phosphorus cannot be over-empahsized, since marginal or deficient levels of these minerals are often used for test purposes.

CHAPTER 8

THE AVAILABILITY OF PHOSPHORUS FROM ANIMAL PROTEIN SOURCES

There is limited information regarding the biological value for the phosphorus in animal protein supplements when fed to chicks. Spandorf and Leong (1964) reported that the biological availability of the phosphorus in twelve menhaden fish meals averaged 99 percent and ranged from 95 to 103 percent of the values obtained with corresponding levels from dicalcium phosphate.

Because of the emphasis placed on linear programming of poultry diets, it is important that accurate information be available regarding the nutritional value of feedstuffs. The present experiments were designed to determine the phosphorus availability of some animal protein sources frequently used in poultry diets.

Experimental Procedure

Samples of fish meal, poultry by-products meal, and meat and bone meal were obtained from several sources. The various samples of the individual ingredients were blended for use in these trials. The blended samples were analyzed for protein, calcium, and phosphorus content by 3 laboratories. The average of these 3 analyses appears in Table 25 and was used in formulation of the test diets. It was recognized that protein quality of the diets could be a confounding factor; therefore,

TABLE 25

Analyses of animal protein sources

Ingredient	Composition[1]		
	% Protein	% P	% Ca
Menhaden fish meal	61.4	2.94	5.27
Poultry by-product meal	58.2	2.01	4.14
Meat and bone meal	53.3	3.52	10.30

[1] Average of analyses by three laboratories.

all diets were formulated to contain 25 percent protein. It was felt that this would help to minimize any possible variation due to protein quality of the diets.

The composition of the basal diets is presented in Table 26. Isolated soybean protein was used to maintain a constant protein level while corn oil and cerelose were used to maintain a constant dietary energy level of 1000 Calories of productive energy per pound. Constant levels of plant phosphorus (0.40 percent) were maintained by variation in degerminated corn meal (0.10 percent P) and white corn meal (0.28 percent P).

Analytical reagent grade monosodium phosphate ($NaH_2PO_4 \cdot H_2O$) and feed grade dicalcium phosphate were used as standards of comparison for phosphorus availability. The test sources were added to the basal diets in amount sufficient to supply 0.05, 0.10 and 0.15 percent phosphorus, giving total phosphorus levels of 0.45, 0.50 and 0.55 percent. These levels are below the minimum phosphorus requirement of the chick as suggested by the National Research Council (1960). The total calcium content of the diet was maintained at 0.80 percent by the use of reagent grade calcium carbonate and the diet supplemented with 1000 I.C.U. per pound of vitamin D_3.

Day-old broiler chicks obtained from a commercial hatchery were randomly assigned to pens in battery brooders. Four replicate pens, each containing 5 male and 5 female chicks, were assigned to each dietary treatment in 2 successive trials, resulting in a total of 80 chicks per treatment.

TABLE 26

Composition of basal diets

Ingredients	Percent of diet			
White corn meal	34.70	36.20	33.20	30.20
Degerminated corn meal	14.00	12.50	15.50	18.50
Soybean meal (50% protein)	32.00	32.00	32.00	32.00
Corn oil	0.73	0.63	0.87	3.28
Cerelose	10.33	10.10	10.33	7.95
Assay protein[1]	3.77	3.34	4.14	4.86
Iodized salt	0.40	0.40	0.40	0.40
Calcium carbonate	1.47	1.54	1.37	1.69
Micro-ingredients[2]	0.90	0.90	0.90	0.90
Monosodium phosphate	0.14	0.14	0.14	0.36
Dicalcium phosphate	. .	. .	. .	. .
Fish meal	1.70	. .	. .	. .
Poultry by-product meal	. .	2.49	. .	. .
Meat and bone meal	. .	. .	1.29	. .
% Protein	25.0	25.0	25.0	25.0
Productive energy Cal/lb.	1000	1000	1000	1000
% Calcium	0.80	0.80	0.80	0.80
% Phosphorus	0.45	0.45	0.45	0.45

[1] C-1 assay protein, Archer-Daniels-Midland Co., Cincinnati, Ohio.

[2] Micro-ingredients as given in Table 5 with 1000 I.C.U. per pound of vitamin D.

Diets and tap water were consumed ad libitum from 1 to 28 days of age at which time the experiment was terminated. Individual body weights were obtained and 2 males and 2 females from each pen were sacrificed for bone ash determination. Since there was no treatment X trial interaction, results of the two trials were combined for presentation.

Results and Discussion

Body weight of chicks fed graded levels of phosphorus from animal protein sources ranged from 95 to 99 percent of that resulting from feeding chicks equivalent levels from the inorganic phosphorus sources (Table 27). Within each level of phosphorus tested, little variation in performance was observed between sources with the exception of the highest level of supplemental phosphorus. It is possible that this may be due to either the quality of the protein or an amino acid imbalance.

Phosphorus supplied from the animal protein sources resulted in tibia ash values that ranged from 101 to 102 percent as much as the inorganic phosphate sources (Table 28). There was little variation observed within each level of phosphorus supplementation.

These data support the findings of Spandorf and Leong (1964) regarding the high availability of phosphorus in fish meal and indicate that the phosphorus in poultry by-product meal and meat and bone meal is highly available for chicks. Therefore, analytical phosphorus values may be used in feed formulation without adjustment for availability.

TABLE 27

Body weight of chicks fed diets supplemented with phosphorus from animal proteins and inorganic phosphate

Phosphorus Source	Added Phosphorus (%)[1] 0.05	0.10	0.15	Av	% Relative Utilization[2]
	Body weight (grams)[3]				
Fish meal	293^{ab}	313^{bcd}	334^{fg}	313	96
Poultry by-products meal	289^{a}	319^{de}	326^{ef}	311	95
Meat and bone meal	293^{ab}	333^{fg}	343^{g}	323	99
Monosodium phosphate	300^{ab}	323^{def}	356^{h}	326	100
Dicalcium phosphate	302^{bc}	323^{def}	355^{h}	326	100

[1] Indicates percent of phosphorus from test source added to basal diet calculated to contain 0.40 percent total phosphorus.

[2] Relative utilization of phosphorus from test source as compared to monosodium phosphate.

[3] Treatment means are average weights of 40 male and 40 female broiler chicks. Means bearing the same superscript do not differ significantly ($P = 0.05$).

TABLE 28

Tibia ash of chicks fed diets supplemented with phosphorus from animal proteins and inorganic phosphate

	Added Phosphorus (%)[1]				
Phosphorus Source	0.05	0.10	0.15	Av	% Relative Utilization[2]
	Tibia Ash (%)[3]				
Fish meal	36.7^{abc}	38.4^{bcd}	41.5^{ef}	38.8	102
Poultry by-products meal	36.2^{ab}	38.9^{cde}	40.6^{def}	38.6	101
Meat and bone meal	36.0^{a}	39.7^{def}	41.6^{f}	39.1	102
Monosodium phosphate	35.7^{a}	39.1^{cde}	39.8^{def}	38.2	100
Dicalcium phosphate	35.2^{a}	38.2^{bc}	41.3^{ef}	38.3	100

[1] Indicates percent of phosphorus from test source added to basal diet calculated to contain 0.40 percent total phosphorus.

[2] Relative availability of phosphorus from test source as compared to monosodium phosphate.

[3] Expressed as percent fat-free, moisture-free bone. Treatment means are averages of 16 male and 16 female broiler chicks. Means bearing the same superscript do not differ significantly ($P = 0.05$).

CHAPTER 9

THE AVAILABILITY OF PHOSPHORUS FROM PLANT SOURCES

The major portion of the phosphorus contained in cereals, cereal by-products, soybeans, and other plant materials is in the form of phytic acid (inositol hexaphosphoric acid) and its salts. Published reports are not in complete agreement concerning the availability of this source of phosphorus. Phosphorus extracted from various plant materials was poorly available for chicks and rats in studies reported by Lowe _et al_. (1939), Krieger _et al_. (1940, 1941), Spitzer _et al_. (1948), Gillis _et al_. (1949, 1957) and Matterson _et al_. (1946). However, the last group stated that one should not infer from the poor availability of the extracted plant phosphate that phosphorus of natural plant material is necessarily unavailable.

The reports of Heuser _et al_. (1943) and McGinnis _et al_. (1944) suggested limited availability of phosphorus from plant sources. Gillis _et al_. (1949) indicated that phosphorus present in natural plant ingredients was a slightly more effective source of phosphorus than that extracted as calcium phytate. Singsen _et al_. (1947) reported that phosphorus in a mixture of cereal grains was relatively unavailable for bone calcification although the addition of vitamin D improved its utilization. Wheat bran phosphorus was poorly utilized by rats in the absence of vitamin D (Boutwell _et al_., 1946). However, an adequate intake of this vitamin increased the utilization of this phosphorus source for bone calcification nearly to that of inorganic phosphorus.

The phosphorus in soybean meal was utilized by rats for both growth and bone formation (Spitzer and Phillips, 1945 a,b). Fritz et al. (1947) noted that under practical conditions cereal grain phosphorus was well utilized by turkey poults. The phosphorus in unifine flour was shown to be almost completely available for growth and somewhat less available for bone deposition in the chick (Sieburth et al., 1952). Vandepopuliere et al. (1961) reported that plant source phosphorus was readily available for growth in chicks when fed at an optimum Ca:P ratio of 1:1. Temperton and Cassidy (1964 a,b) concluded from balance studies that the chick was able to absorb and retain a large proportion of ingested plant phosphorus, and utilize this phosphorus for deposition in growing bones. This supports earlier work by Singsen et al. (1950) in which it was demonstrated by P^{32} tracer studies that phytin phosphorus can move freely about the body and participate in any reaction requiring phosphorus.

The utilization of this organically bound form of phosphorus is of considerable practical importance since most poultry diets must be supplemented with phosphorus for optimum performance. Therefore, it was considered important to determine the effect of the source or form of the organic phytin phosphorus and the influence of dietary factors upon its availability to the chick in order to estimate the extent to which plant phosphorus can be used to meet the phosphorus requirement.

The Availability of Phytic Acid Phosphorus for Chicks

Since the report of Vandepopuliere et al. (1961) indicated that plant source phosphorus was readily available to support growth in chicks when fed at narrow Ca:P ratios, this trial was conducted to determine the availability of phytic acid phosphorus and the possible effect of Ca:P ratio upon its availability.

Experimental Procedure

Two trials were conducted with day-old broiler chicks. Graded levels of phosphorus from phytic acid (0.05 to 0.40 percent) and dicalcium phosphate (0.05 to 0.20 percent) were fed to chicks at Ca:P ratios of 1:1 and 2:1 (Table 29). The basal diets contained 0.27 percent total phosphorus.

The phytic acid was supplied in a solution containing 70 percent phytic acid. All experimental diets were kept iso-caloric and iso-nitrogenous by varying the amount of corn, soybean oil meal and animal fat. Increased calcium content was obtained by increasing the level of ground limestone.

Two replications of 5 males and 5 females were used per treatment in each trial in battery brooders. Experimental diets and tap water were given ad libitum.

Individual body weights of chicks were obtained at 4 weeks of age. Since the diet X trial interaction was not significant, only average weights from the combined trials are given. Three males and 3 females from each pen in trial 1 were sacrificed for bone ash determination.

TABLE 29

Composition of basal diets

Ingredients	Ca:P Ratio 1:1	Ca:P Ratio 2:1
	Percent of diet	
Degerminated corn meal	64.38	63.20
Soybean meal (50% protein)	31.20	31.28
Animal fat	. .	0.35
Ground limestone	0.32	1.07
Alfalfa meal (17% portein)	3.00	3.00
Micro-ingredients[1]	0.70	0.70
Iodized salt	0.40	0.40

[1] Micro-ingredients as outlined in Table 5.

Results and Discussion

Growth rate and tibia ash of chicks were significantly increased with each increase of dietary phosphorus supplied either by phytic acid or dicalcium phosphate (Table 30).

Growth rate and tibia ash of chicks receiving supplementary phosphorus from phytic acid were comparable to chicks receiving equivalent levels of phosphorus from dicalcium phosphate. These data are in disagreement with the earlier findings (Lowe et al., 1939; Heuser et al., 1943; McGinnis et al., 1944; and Gillis et al., 1949) that phytin phosphorus could be utilized slightly, if at all, by the chick. However, these data are in agreement with an earlier report by Vandepopuliere et al. (1961) and agree with data obtained from feeding rats, where phytic acid or phytin phosphorus was available for growth and bone calcification (Krieger et al., 1940; Spitzer et al., 1948).

The phosphorus source X Ca:P ratio interaction for body weight and bone ash was found to be statistically significant ($P = .01$). This significant interaction was a result of slightly lowered body weights or bone ash of chicks fed phytic acid when the Ca:P ratio was widened as compared to an increased body weight or bone ash when the ratio was widened with dicalcium phosphate as a phosphorus source. That widening the Ca:P ratio interferes with utilization of phytin phosphorus agrees with the suggestion of Vandepopuliere et al. (1961). However, it does not explain the high availability of the phytic acid phosphorus in this study as compared to earlier reports with the chick.

TABLE 30

Body weight and tibia ash of chicks fed various levels of phosphorus from phytic acid and dicalcium phosphate

Supplement		1:1 Ca:P ratio		2:1 Ca:P ratio	
% P added	Source	Body wt. (g)	Tibia ash (%)	Body wt. (g)	Tibia ash (%)
0[1]	. .	279	28.8	279	27.5
.05	Phytic acid	308	31.1	297	27.0
.10	Phytic acid	346	32.2	344	28.1
.20	Phytic acid	362	35.1	359	35.2
.40	Phytic acid	381	37.5	. .	. .
.05	Dicalcium phosphate	270	30.6	328	27.3
.10	Dicalcium phosphate	318	31.7	366	29.5
.20	Dicalcium phosphate	354	35.4	402	39.6

[1] Basal diet contained 0.27 percent phosphorus.

It has been shown that heat treatment increases the availability of zinc in soybean protein (Supplee et al., 1958; and Kratzer et al., 1959), and an interrelationship has been shown between the zinc content of the diet and utilization of phytic acid (O'Dell et al., 1961). Since soybean oil meals are now prepared in a different manner than those used in the earlier work, this difference and supplementary zinc in the basal diet may be the factors responsible for the high availability of the phosphorus from phytic acid.

Comparison of Phytin Phosphorus Sources

Results of the previous studies indicated that the phosphorus in a solution of phytic acid was available to the chick for growth and bone calcification. This series of trials was conducted to determine the phosphorus availability of salts of phytic acid.

Experimental Procedure

Three trials were conducted to determine the availability of phosphorus as phytic acid, calcium-magnesium phytate, and sodium phytate. The phytic acid* (inositol hexaphosphoric acid) contained 28.16 percent phosphorus. The supplement used was an aqueous solution containing 70 percent phytic acid. The calcium phytate,** described as phytic acid calcium-magnesium salt with an approximate analysis of 12 percent calcium, 1.5 percent magnesium and 22 percent phosphorus, was in the form of a white, odorless powder, insoluble in water. The sodium phytate,* described as a neutral phytic acid sodium salt, contained 23.3 percent phosphorus. It was a white powder which was completely soluble in water.

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*Nutritional Biochemicals Corp., Cleveland Ohio.

**Corn Products Co., New York, N. Y.

A practical type diet, using degerminated corn as the major energy source and dehulled soybean meal as the major protein source, served as the basal diet for all trials (Table 31). This diet was calculated to contain 0.30 percent phosphorus, all from plant sources, 20 percent protein, and 1,000 kilocalories of productive energy per pound.

Day-old broiler chicks were randomly assigned to pens in battery brooders. The experimental feeds and tap water were supplied ad libitum. Three pens, each containing 5 male and 5 female chicks, were assigned to each of the experimental treatments in all trials.

Trial 1.--Feed grade dicalcium phosphate, calcium phytate, and phytic acid were added to the basal diet to supply 0.10 and 0.20 percent supplemental phosphorus, resulting in total phosphorus levels of 0.40 and 0.50 percent. A constant 2:1 ratio of calcium to phosphorus was maintained by the addition of reagent grade calcium carbonate. Diets were maintained iso-caloric by the addition of finely ground oat hulls. Chicks were placed on the experimental diets at 1 day of age and maintained for a 28-day feeding period, at which time individual body weights were obtained and 2 male and 2 female chicks from each pen were sacrificed for bone ash determination.

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*Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE 31

Composition of basal diet

Ingredient	Percent
Degerminated corn	56.70
Soybean meal (50% protein)	34.00
Alfalfa meal (17% protein)	3.00
Iodized salt	0.40
Micro-ingredients[1]	0.90
Variable ingredients[2]	5.00

[1] Micro-ingredients as outlined in Table 5.

[2] Consisted of calcium carbonate, phosphorus source and oat hulls in amounts calculated to attain desired calcium and phosphorus levels.

Trial 2.--The basal diet (Table 31) was supplemented with 0.10 or 0.20 percent phosphorus from either phytic acid, calcium phytate, sodium phytate, or monosodium phosphate ($NaH_2PO_4 \cdot H_2O$). This resulted in total phosphorus levels of 0.40 and 0.50 percent. The calcium level of all diets was maintained at 0.60 percent total calcium with 340 I.C.U. of vitamin D_3 per pound of feed. The experimental diets and tap water were supplied *ad libitum*. Individual body weights were determined at 28 days of age.

Trial 3.--The basal diet (Table 31) was supplemented with 0.10, 0.20 and 0.40 percent phosphorus from phytic acid, calcium phytate, sodium phytate, and monosodium phosphate to give total phosphorus levels of 0.40, 0.50 and 0.70 percent. The calcium level of all diets was maintained at 0.60 percent total calcium by the addition of calcium carbonate.

All diets were supplemented with 360 I.C.U. of vitamin D_3 per pound. In addition, diets supplemented with 0.40 percent phosphorus from each of the 4 phosphorus sources were supplemented with 1,080 I.C.U. of vitamin D_3 per pound to determine the effects of increased vitamin D levels on the availability of the phosphorus from the various phytin sources.

Since the report of O'Dell *et al.* (1961) indicated an interaction between calcium and phytic acid relative to the availability of zinc, it seemed desirable to determine the effects of zinc supplementation of the basal diet upon the availability of the phosphorus from the phytin sources. Therefore, the diets containing 360 I.C.U. of vitamin D_3 per pound and 0.40

percent added phosphorus from each of the 4 sources were supplemented with 200 p.p.m. of zinc in the form of zinc sulfate and compared to comparable diets without zinc supplementation. The experimental diets and tap water were supplied *ad libitum*. At 28 days of age individual body weights were obtained.

Results and Discussion

Trial 1.--No significant differences were observed between the availability of phosphorus from phytic acid or dicalcium phosphorus as determined by body weight or tibia ash (Table 32). This was true at both levels of phosphorus supplementation. The addition of 0.10 percent phosphorus from calcium phytate to the basal diet significantly increased both body weight and bone ash but the response was not comparable to that of phytic acid or dicalcium phosphate at this level of supplementation. Increasing the level of phosphorus supplementation to 0.20 percent from calcium phytate resulted in a slight but non-significant increase in body weight as compared to the basal diet, but a decrease as compared to the 0.10 level. Tibia ash was significantly increased, however, lending support to the report by Sieburth *et al.* (1952) that the phosphorus from calcium phytate was relatively unavailable for growth but available for bone deposition. In no case, however, did the degree of calcification on the calcium phytate diets approach that attained on the phytic acid or dicalcium phosphate diets.

Trial 2.--Diets supplemented with phosphorus from calcium phytate did not support a level of growth equivalent to that obtained from feeding

TABLE 32

Body weight and tibia ash of chicks fed diets supplemented with various sources of organic and inorganic phosphorus

Phosphorus		Body weight (gms.)[1]			Tibia ash (%)[1]		
Source	% Added	M	F	Av	M	F	Av
Basal diet[2]	0	211	200	205^{d}	23.5	25.1	24.3^{k}
Calcium phytate	0.10	287	247	267^{c}	26.3	26.6	26.5^{j}
	0.20	224	232	228^{d}	25.5	29.2	27.9^{i}
Phytic acid	0.10	353	323	338^{b}	30.6	32.8	31.7^{h}
	0.20	393	342	368^{a}	39.9	39.0	39.5^{f}
Dicalcium phosphate	0.10	365	310	338^{b}	32.4	30.8	31.6^{h}
	0.20	388	346	367^{a}	35.9	38.2	37.0^{g}

[1] Means bearing the same superscripts do not differ significantly (P = 0.05).

[2] Basal diet calculated to contain 0.30% phosphorus. All diets fed at a 2:1 Ca:P ratio.

the other phosphorus sources at either level of supplementation (Table 33). Sodium phytate phosphorus at the 0.10 percent level of supplementation supported a growth rate that was numerically but not significantly superior to the calcium phytate groups. When the sodium phytate supplementation was increased to 0.20 percent phosphorus, body weight data indicated its utilization was significantly greater than that of calcium phytate but did not approach that of the phytic acid or monosodium phosphate supplements. Body weight data indicated that phytic acid phosphorus was not as available as the monosodium phosphate at the 0.10 percent level of supplementation but was equally available at the 0.20 percent level.

Trial 3.--Comparison of the growth rate of chicks fed the phosphorus supplements at a level of 0.10 percent added phosphorus indicated monosodium phosphate to be superior to the three phytin phosphorus sources (Table 34). Phytic acid and sodium phytate were of equal value in promoting chick growth, while calcium phytate was inferior to all phosphorus sources tested. When the phosphorus supplementation was increased to 0.20 percent, phytic acid supported a growth rate comparable to that of chicks on diets supplemented with monosodium phosphate. Supplementation with sodium phytate at this level did not support a growth rate comparable to that of monosodium phosphate or phytic acid supplementation, but was superior to calcium phytate supplementation. Calcium phytate continued to give inferior performance; however, a significant improvement in growth was obtained as the level of phosphorus supplementation from this source was increased from 0.10 to 0.20 percent.

TABLE 33

Body weight of chicks fed diets supplemented with various sources of organic and inorganic phosphorus

	% Added phosphorus[1]					
	0.10			0.20		
Phosphorus source	M	F	Av	M	F	Av
	Body weight (grams)[2]					
Phytic acid	386	338	361^{fg}	442	406	423^{i}
Sodium phytate	351	341	345^{ef}	404	364	383^{gh}
Calcium phytate	349	327	337^{de}	331	303	317^{d}
Monosodium phosphate	435	378	406^{hi}	446	391	418^{i}

[1] The basal diet was calculated to contain 0.30 percent phosphorus, all from plant sources. All diets contained 0.60 percent calcium.

[2] Means bearing the same superscript do not differ significantly ($P = 0.05$).

TABLE 34

Body weight of chicks fed diets supplemented with various sources of organic and inorganic phosphorus

Phosphorus		– Zinc						Zinc[1]		
Source	% Added	360 D_3			1,080 D_3			360 D_3		
		M	F	Av	M	F	Av	M	F	Av
		Body weight (grams)[2]								
Basal[3]	0	356	245	300^{a}	410	350	380^{cd}	316	274	295^{a}
Monosodium phosphate	0.10	430	392	411^{ef}	. .	. .	. .	. .	. .	. .
	0.20	465	421	443^{ghi}	. .	. .	. .	. .	. .	. .
	0.40	478	434	456^{hi}	483	427	458^{hi}	489	445	467^{i}
Phytic acid	0.10	373	350	362^{bc}	. .	. .	. .	. .	. .	. .
	0.20	475	415	445^{hi}	. .	. .	. .	. .	. .	. .
	0.40	466	428	447^{hi}	472	417	445^{hi}	479	435	457^{hi}
Sodium phytate	0.10	395	345	370^{bc}	. .	. .	. .	. .	. .	. .
	0.20	398	370	384^{cd}	. .	. .	. .	. .	. .	. .
	0.40	461	428	444^{ghi}	440	396	418^{efg}	449	422	435^{fgh}
Calcium phytate	0.10	362	277	320^{a}	. .	. .	. .	. .	. .	. .
	0.20	361	335	348^{b}	. .	. .	. .	. .	. .	. .
	0.40	392	367	360^{c}	424	381	403^{de}	371	355	363^{bc}

[1] Zinc supplementation at 200 p.p.m. supplied by zinc sulfate.

[2] Means bearing the same superscript do not differ significantly ($P = 0.05$).

[3] The basal diet was calculated to contain 0.30 percent phosphorus. All diets contained 0.60 percent calcium.

When the level of phosphorus supplementation was increased to 0.40 percent, monosodium phosphate, phytic acid, and sodium phytate supported an equal rate of growth. Calcium phytate supplementation at this level significantly increased growth rate as compared to the 0.20 percent level of supplementation, but performance was inferior to the other three sources.

Increasing the vitamin D_3 level of the diet from 360 to 1,080 I.C.U. per pound at the 0.40 percent level of phosphorus supplementation did not influence chick performance on the diets supplemented with monosodium phosphate, phytic acid, or sodium phytate, but significantly increased the 28-day body weight of chicks receiving supplemental phosphorus from calcium phytate (Table 34).

Zinc supplementation of the diets containing 0.40 percent phosphorus from each of the four sources had no significant effect on 28-day body weight (Table 34).

The results of these trials indicate that the phosphorus from the phytic acid supplement (inositol hexaphosphoric acid) was highly available and equal to the dicalcium or monosodium phosphates in most instances, as measured by body weight and tibia ash. This confirms the report of Harms _et al._ (1962) that phosphorus from phytic acid was as available as that from dicalcium phosphate.

The availability of phosphorus from sodium phytate was somewhat lower than from phytic acid or the inorganic phosphates. The availability of the sodium phytate phosphorus tended to increase as the level of supplementation increased, approaching that of the dicalcium or monosodium phosphate at high levels of supplementation.

The phosphorus in the calcium-magnesium salt or phytic acid (calcium phytate) was essentially unavailable for growth and only slightly available for bone calcification under the conditions of these experiments. This confirms the reports of Matterson _et al._ (1946), Sieburth _et al._ (1952) and Gillis _et al._ (1957). In contrast to the sodium phytate supplement, the availability of the calcium phytate phosphorus appeared to decline as the level of supplementation increased. In only one of three experiments did higher levels of calcium phytate continue to improve performance.

Increasing the vitamin D_3 level of the diet from 360 to 1,080 I.C.U. per pound significantly increased the availability of the calcium phytate phosphorus, but did not influence the availability of phytic acid or sodium phytate phosphorus. This finding agrees with the reports of several workers (Krieger and Stenbock, 1940; Boutwell _et al._, 1946; Singsen _et al._, 1947; Spitzer _et al._, 1948; Gillis _et al._, 1957) and may be due either to its well-known action on calcium absorption or to some activating action on the intestinal phytase enzyme. However, Krieger _et al._ (1940) reported that the calcium from calcium phytate was as available as from calcium carbonate when adequate phosphorus was supplied from inorganic phosphates, and Spitzer _et al._ (1948) reported that vitamin D was not necessary for phytase formation. In both of these tests, weanling rats were used and a species difference may be present.

The findings which indicate high availability for phosphorus in phytic acid and sodium phytate appear to be a direct contradiction of the results of Harrison and Mellanby (1939). These authors reported that phytic acid and sodium phytate exert powerful rachitogenic actions when added to low calcium non-rachitogenic diet while calcium phytate was slightly antirachitic. These workers postulated that this rachitogenic action was due to an inhibition of calcium absorption from the alimentary canal; again, a species difference may be involved as these authors utilized young puppies as their experimental animal.

Effect of Calcium and Vitamin D_3 Levels on the Utilization of Calcium Phytate

Results of the previous studies demonstrated that phosphorus from phytic acid and dicalcium phosphate were of equal availability to the chick. However, a widening of the calcium:phosphorus ratio decreased the relative availability of the phosphorus in phytic acid to a greater degree than phosphorus from dicalcium phosphate.

The phosphorus from the calcium salt of phytic acid was shown to be less available than that from phytic acid or sodium phytate phosphorus. In view of these results, trials were conducted to further define the influence of calcium and vitamin D_3 levels on the utilization of calcium phytate phosphorus.

Experimental Procedure

A practical type diet using degerminated corn meal as the major energy source and dehulled soybean meal as the major protein source served as the basal diet (Table 31). This diet was analyzed to contain 0.30 percent phosphorus (all from plant sources), and was calculated to contain 20 percent protein, and 1,000 kilo-calories of productive energy per pound of feed.

Day-old broiler chicks were randomly assigned to pens in battery brooders. Three pens, each containing 5 male and 5 female chicks, were assigned to each of the experimental treatments. The experimental feeds and tap water were supplied ad libitum.

Trial 1.--Calcium phytate was compared to dicalcium phosphate at levels of 0.05, 0.10, 0.20, and 0.30 percent added phosphorus at calcium to total phosphorus ratios of 0.8:1, 1.4:1, and 2:1. A level of 360 I.C.U. of vitamin D_3 per pound was used in each of these diets. In addition, vitamin D_3 (1,440 I.C.U. per pound of feed) was added to diets supplemented with 0.20 percent phosphorus from calcium phytate at each of the 3 calcium:phosphorus ratios.

The calcium phytate supplement,* described as phytic acid calcium-magnesium salt with an analysis of 12 percent calcium, 1.5 percent magnesium, and 22 percent phosphorus, was in the form of a white odorless powder insoluble in water.

At 21 days of age the chicks were individually weighed and 2 male and 2 female chicks from each pen were sacrificed for bone ash determination.

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*Corn Products Co., New York, N. Y.

Trial 2.--A 2^4 factorial arrangement of 2 phosphorus sources (calcium phytate vs. dicalcium phosphate), 2 supplemental phosphorus levels (0.10 vs. 0.20 percent), 2 calcium levels (0.50 vs. 1.00 percent), and 2 vitamin D_3 levels (360 vs. 6,000 I.C.U. per pound) was used in this test. The chicks and the basal diet were prepared as in Trial 1. The experimental diets were fed for 21 days at which time individual body weights were obtained.

Results and Discussion

Trial 1.--Twenty-one-day body weight and tibia ash were significantly affected by the source and level of phosphorus, the Ca:P ratio and the vitamin D_3 level of the diet (Tables 35 and 36). As expected, there were significant interactions among the factors of phosphorus source, level of added phosphorus, and the calcium:phosphorus ratio of the experimental diets. At the lowest Ca:P ratio, (0.8:1), the addition of 0.05 or 0.10 percent phosphorus from calcium phytate increased body weight and tibia ash, with the increase from the 0.10 percent level statistically significant. A further increase in phosphorus from this source had no additional effect. In fact the addition of 0.30 percent phosphorus from calcium phytate resulted in body weight and tibia ash values similar to those observed on the basal diet with no added phosphorus. In comparison, levels of dicalcium phosphate up to 0.30 percent added phosphorus significantly increased body weight and tibia ash.

A comparison of the 2 sources at the 0.8:1 Ca:P ratio indicates that, with the exception of the diet containing 0.30 percent added

TABLE 35

Body weight of chicks fed different phosphorus sources, levels of vitamin D_3, and calcium:phosphorus ratios

Phosphorus		Vit D I.C.U./lb.	Calcium:phosphorus ratio			
Source	% Added		0.8:1	1.4:1	2:1	Average
			Body weight (grams)[1]			
Basal[2]	0	360	238^{fg}	262^{ij}	202^{c}	234
Calcium phytate	0.05	360	251^{gh}	229^{ef}	184^{b}	221
	0.10	360	281^{lm}	238^{fg}	186^{b}	235
	0.20	360	279^{kl}	213^{cd}	163^{a}	218
	0.20	1,440	284^{mn}	268^{kl}	175^{ab}	242
	0.30	360	235^{fg}	220^{de}	160^{a}	205
Dicalcium phosphate	0.05	360	264^{jk}	267^{kl}	228^{ef}	255
	0.10	360	272^{kl}	274^{kl}	259^{hi}	278
	0.20	360	274^{kl}	300^{no}	285^{mn}	275
	0.30	360	315^{o}	299^{no}	297^{mn}	304

[1] Means bearing the same superscripts do not differ significantly ($P = 0.05$).

[2] Basal diet contained 0.30 percent phosphorus.

TABLE 36

Tibia ash of chicks fed different phosphorus sources, vitamin D_3 levels, and calcium:phosphorus ratios

Phosphorus		Vit D	Calcium:phosphorus ratio			
Source	% Added	I.C.U./lb.	0.8:1	1.4:1	2:1	Average
			Tibia ash (%)[1]			
Basal[2]	0	360	30.9^{cd}	29.4^{bc}	25.9^{a}	28.7
Calcium phytate	0.05	360	33.3^{de}	27.5^{ab}	26.5^{a}	29.1
	0.10	360	33.8^{e}	26.5^{a}	25.7^{a}	28.8
	0.20	360	34.4^{ef}	25.3^{a}	27.6^{ab}	29.1
	0.20	1,440	33.4^{de}	33.5^{e}	27.6^{ab}	31.5
	0.30	360	29.5^{bc}	25.1^{a}	25.2^{a}	26.6
Dicalcium phosphate	0.05	360	34.2^{ef}	29.9^{bc}	25.3^{a}	29.8
	0.10	360	35.0^{ef}	35.0^{ef}	31.2^{cd}	33.8
	0.20	360	36.5^{fg}	39.8^{hi}	37.7^{gh}	38.0
	0.30	360	37.9^{gh}	41.3^{i}	41.0^{i}	40.1

[1] Means bearing the same superscript do not differ significantly ($P = 0.05$).

[2] Basal diet contained 0.30 percent phosphorus.

phosphorus from calcium phytate, there were no significant differences in body weight or tibia ash at comparable phosphorus levels. In several instances, body weight was numerically greater for the calcium phytate groups (Table 35), while dicalcium phosphate tended to produce a greater degree of bone calcification (Table 36).

An increase in the Ca:P ratio to 1.4:1 significantly depressed body weight and tibia ash of the chicks fed diets supplemented with phosphorus from calcium phytate. A further increase in the Ca:P ratio to 2:1 severely lowered body weights but had little additional effect on bone ash of chicks consuming diets supplemented with calcium phytate. Chicks fed comparable diets supplemented with phosphorus from dicalcium phosphate were not as severely affected by increases in the Ca:P ratio. This was observed as a source X ratio interaction that was highly significant for both body weight and tibia ash.

At the 2:1 Ca:P ratio, higher levels of added phosphorus from calcium phytate (i.e., 0.20 and 0.30 percent) produced a more severe depression in body weight than did lower levels. However, this effect was not as severe when tibia ash was the criterion. Chicks fed the diets containing dicalcium phosphate tolerated the 2:1 Ca:P ratio to a greater extent than did chicks fed the calcium phytate supplemented diets. As the level of phosphorus from dicalcium phosphate increased, less depression in body weight or tibia ash was observed.

Increasing the level of vitamin D_3 (1,440 vs. 360 I.C.U./lb.) in the diet supplemented with 0.20 percent phosphorus from calcium phytate significantly improved body weight and tibia ash at a Ca:P ratio of 1.4:1 (Tables 34 and 35). At Ca:P ratios of 0.8:1 and 2:1, body weight was numerically but not significantly increased by the addition of the higher level of vitamin D_3. However, little effect was observed on tibia ash at these Ca:P ratios.

Trial 2.--Calcium phytate was inferior to dicalcium phosphate as a phosphorus source at all levels of phosphorus, calcium and vitamin D_3 supplementation as measured by body weight (Table 37). The availability of both sources was significantly affected by alterations in the calcium and vitamin D_3 levels in the diet.

At either level of calcium supplementation, increasing the vitamin D_3 level of the diet from 360 to 6,000 I.C.U. per pound resulted in an increase in 21-day body weight. This increase was statistically significant at all levels of supplementation with calcium phytate and at the 0.10 percent phosphorus level of supplementation with dicalcium phosphate. The increase in body weight resulting from the increased vitamin D_3 supplementation at higher levels of supplementation with dicalcium phosphate was numerically superior at the 1.0 percent level of calcium and significantly superior at the 0.5 percent level of calcium.

Chicks fed diets supplemented with phosphorus from calcium phytate were heavier when the diet contained 0.5 percent calcium than at 1.0 percent calcium. Diets containing 0.10 percent phosphorus from dicalcium phosphate also supported greater growth at the lower calcium level,

TABLE 37

Body weight of chicks fed different phosphorus sources with varying levels of calcium and vitamin D_3

Phosphorus		1% Calcium		0.5% Calcium	
Source	% Added	360	6,000	360	6,000
			Body weight (grams)[1]		
Basal diet[2]	0	114^{a}	208^{d}	. .	. .
Calcium phytate	0.10	129^{b}	227^{efg}	206^{d}	251^{hi}
	0.20	134^{b}	217^{de}	217^{de}	268^{j}
Dicalcium phosphate	0.10	218^{e}	241^{gh}	276^{j}	294^{k}
	0.20	265^{ij}	277^{j}	237^{fgh}	298^{kl}

[1] Means bearing the same superscript do not differ significantly ($P = 0.05$).

[2] Basal diet contained 0.30 percent phosphorus.

but when the supplemental phosphorus level was increased to 0.20 percent from dicalcium phosphate, growth on the higher calcium level was favored when both levels of supplemental vitamin D_3 were combined. However, the maximum growth at this level of phosphorus supplementation resulted when the calcium level of the diet was 0.50 percent and the vitamin D_3 level was 6,000 I.C.U. per pound.

The results of these trials demonstrate a close relationship between calcium and phosphorus levels and ratios and the vitamin D_3 level of the diet upon the utilization of the phosphorus from calcium phytate. Low calcium levels or low Ca:P ratios tended to improve the availability of the calcium phytate phosphorus. In several instances the improvement in phosphorus availability was such that calcium phytate phosphorus was equal to dicalcium phosphate phosphorus in promoting growth or bone calcification. These low calcium levels, however, were not the most desirable levels for optimum performance on diets supplemented with dicalcium phosphate. Higher levels of calcium or wider Ca:P ratios were required for optimum performance on dicalcium phosphate supplemented diets; these higher calcium levels lowered the availability of the calcium phytate phosphorus.

Increasing the level of vitamin D_3 in the diet (1,440 to 6,000 vs. 360 I.C.U./lb. of feed) improved the utilization of phosphorus from both calcium phytate and dicalcium phosphate at certain Ca:P levels and ratios. This would be expected since the data in Chapter 7 demonstrated that the vitamin D_3 requirement of chicks was increased at unfavorable calcium:phosphorus ratios.

The Availability of Natural Plant Phosphorus

The majority of the workers who have reported a low availability for organic phosphorus have utilized a chemically isolated product, rather than the intact form. Results of the previous experiment demonstrated a significant difference in the availability of various salts of phytin phosphorus. Therefore, this study was conducted to determine the availability of phosphorus in various portions of the corn grain without chemical isolation or extraction.

Experimental Procedure

Three types of corn products were used as primary energy sources in a simplified chick feed to study the availability of plant phosphorus. These products were ground whole white corn, degerminated white corn meal, and hominy meal. The hominy meal is a by-product of corn meal manufacture and consists of a mixture of the corn bran, the corn germs, and a part of the starchy portion of the kernels. It is fully equal to corn in poultry diets (Morrison, 1959). All 3 ingredients were submitted to 3 laboratories for phosphorus analysis (Table 38).

Basal diets were prepared using each of the 3 corn products (Table 39). A constant amount of soybean meal was used in order that the variation in phosphorus level of the diet would be a result of the corn component. Some adjustment of the hominy meal diet was necessary to maintain a constant level of energy and protein. Cerelose and corn oil were added to adjust for energy content and isolated soy protein

TABLE 38

Phosphorus analysis of corn products

Corn Product	Laboratory 1	Laboratory 2	Laboratory 3	Ave.
	% Phosphorus			
Degerminated corn	0.09	0.09	0.11	0.10
Corn meal	0.26	0.25	0.28	0.26
Hominy meal	0.61	0.63	0.59	0.61

TABLE 39

Composition of diets

Ingredient	Percent of Diet 1	2	3
Degerminated corn	59.7	. .	. .
White corn meal	. .	59.7	. .
Hominy meal	. .	. .	41.0
Corn oil	. .	. .	4.45
Cerelose	. .	. .	13.34
Soy protein	. .	. .	0.91
Iodized salt	0.40	0.40	0.40
Soybean meal (50% protein)	34.00	34.00	34.00
Micro-ingredients[1]	0.90	0.90	0.90
Variable[2]	5.00	5.00	5.00
% Protein	22.40	22.40	22.40
Calories PE/lb.	928.00	928.00	928.00
% P	0.30	0.40	0.50
% Ca	0.60	0.60	0.60

[1] Micro-ingredients as outlined in Table 5.

[2] Consisted of reagent grade calcium carbonate, reagent grade monosodium phosphate, and pulverized oat hulls.

added to adjust protein levels. Using an average of the analytical values for the 3 ingredients, the diets were calculated to contain 0.30, 0.40, and 0.50 percent phosphorus for the degerminated corn, white corn meal, and hominy meal diets, respectively. Chemical analyses of the mixed diets by the 3 laboratories were in close agreement with calculated values.

To each of the 3 basal diets, graded levels of inorganic phosphorus were added in the form of reagent grade monosodium phosphate ($NaH_2PO_4 \cdot H_2O$). Phosphorus supplementation levels were o, 0.10, 0.20, 0.30, and 0.40 percent, resulting in a 3 X 5 factorial arrangement of treatments. The total calcium content of all diets was held constant at 0.60 percent by the addition of calcium carbonate. This level of calcium supplementation met the calcium requirement of the 0- to 4-week-old chick for this basal diet (Chapter 6) without creating a severe Ca:P inbalance in the diet. The supplemental vitamin D level was 660 I.C.U. per pound of feed.

Three successive feeding trials were conducted, using the original samples of the corn products to prepare the feeds for each trial. Day-old broiler chicks were randomly assigned to pens in battery brooders. The experimental feeds and tap water were offered *ad libitum*. Ten chicks, equally divided as to sex, were assigned to each pen. In each of the 3 feeding trials, 4 pens were assigned to each dietary treatment, resulting in an over-all total of 120 chicks per treatment.

At 28 days of age the chicks were individually weighed and 2 chicks of each sex were sacrificed from each pen for bone ash determination.

Analysis of the data indicated no significant trial X treatment interaction and permits discussion of the average results of the 3 trials.

Results and Discussion

Organic plant phosphorus, supplied in the diet as corn meal or hominy meal, was utilized as well as inorganic phosphorus in promoting body weight gains (Table 40). However, it was somewhat less available for calcification of bones (Table 41). As there was no sex X treatment interaction observed, discussion is based on treatment average.

Examination of the body weight data presented in Table 40 indicates that 0.10 percent organic plant phosphorus furnished by corn meal promoted significantly greater growth than 0.10 percent inorganic phosphorus added to a low-phosphorus basal diet composed of degerminated corn. However, in comparison to the low-phosphorus degerminated corn basal diet supplemented with 0.2 or 0.3 percent inorganic phosphorus, the 0.1 percent level of added organic phosphorus from corn meal supported significantly less body weight gains in diets having identical levels of total phosphorus. At total phosphorus level of 0.70 percent, the 0.1 percent organic phosphorus furnished by corn meal again significantly improved growth rate as compared to the low-phosphorus degerminated corn supplemented with inorganic phosphorus.

Organic phosphorus supplied from hominy meal also supported body weight gains. A significant improvement in body weight was obtained when a basal diet containing 0.50 percent organic phosphorus using hominy meal was compared to 0.2 percent inorganic phosphorus supplementation of the

TABLE 40

Body weight of broiler chicks fed diets with plant phosphorus from three sources

Plant Phosphorus Source	Organic Phosphorus (%)[1]	Sex	Total Phosphorus (%)						
			0.30	0.40	0.50	0.60	0.70	0.80	0.90
			Body Weight (grams)[2]						
Degerminated corn	0.30	M	294	336	373	412	395	. .	. .
		F	263	280	337	371	361	. .	. .
		Av.	278^{a}	308^{b}	355^{e}	392^{hij}	378^{gh}	. .	. .
Corn meal	0.40	M	. .	331	353	373	412	381	. .
		F	. .	315	320	342	375	362	. .
		Av.	. .	323^{c}	337^{d}	357^{e}	394^{ij}	372^{fg}	. .
Hominy meal	0.50	M	. .	. .	432	396	397	388	396
		F	. .	. .	372	373	367	350	354
		Av.	. .	. .	402^{j}	385^{ghi}	382^{ghi}	369^{ef}	375^{fg}

[1] Values indicate the amount of organic phosphorus supplied by the all-vegetable diets utilizing the three sources of plant phosphorus. The remainder of the total dietary phosphorus is supplied by monosodium phosphate ($NaH_2PO_4H_2O$).

[2] Means bearing the same superscript do not differ significantly ($P = 0.05$).

degerminated corn diet to give a total phosphorus level of 0.50 percent. At higher levels of total dietary phosphorus, however, the organic phosphorus from hominy meal was as effective in supporting body weight as that from the inorganic phosphorus source.

The organic phosphorus from either corn meal or hominy meal was significantly less available for bone calcification than inorganic phosphorus (Table 41). When no supplemental inorganic phosphorus was added to the 3 all-plant diets, 0.1 percent additional organic phosphorus furnished by corn meal and 0.2 percent additional organic phosphorus from hominy meal significantly improved calcification as compared to the degerminated corn meal diet. No significant difference was observed between the corn meal (0.4 percent total phosphorus) and the hominy meal (0.5 percent total phosphorus) basal diets.

Addition of inorganic phosphorus to the degerminated corn diet produced tibia ash values that were equal to or significantly superior to corn meal or hominy meal diets containing equivalent levels of total dietary phosphorus but with greater amounts of organic phosphorus.

The data from these studies demonstrate that organic plant phosphorus supported body weight gains equal to those obtained with inorganic phosphorus when the total dietary phosphorus level was below the minimum phosphorus requirement of the chick. At higher phosphorus levels there were few significant differences observed between any of the treatment groups.

In contrast to the body weight data, the organic plant phosphorus was considerably less available for bone calcification than was inorganic

TABLE 41

Tibia ash of broiler chicks fed diets with plant phosphorus from three sources

Plant Phosphorus Source	Organic Phosphorus (%)[1]	Sex	Total Phosphorus (%) 0.30	0.40	0.50	0.60	0.70	0.80	0.90
			Tibia ash (%)[2]						
Degerminated corn	0.30	M	28.4	32.1	37.8	38.5	39.9	. .	. .
		F	29.1	31.6	37.6	38.3	39.1	. .	. .
		Av.	28.7^{a}	31.9^{b}	37.7^{de}	38.4^{ef}	39.5^{f}	. .	. .
Corn meal	0.40	M	. .	30.1	31.8	36.8	37.3	36.3	. .
		F	. .	33.4	33.7	38.1	38.0	39.3	. .
		Av.	. .	31.7^{b}	32.8^{b}	37.5^{de}	37.6^{de}	37.8^{de}	. .
Hominy meal	0.50	M	. .	. .	31.0	34.0	35.9	35.4	35.4
		F	. .	. .	31.7	35.6	36.8	36.4	36.5
		Av.	. .	. .	31.4^{b}	34.8^{c}	36.3^{cd}	35.9^{c}	35.9^{c}

[1] Values indicate the amount of organic phosphorus supplied by the all-vegetable diets utilizing the three sources of plant phosphorus. The remainder of the total dietary phosphorus is supplied by monosodium phosphate ($NaH_2PO_4H_2O$).

[2] Means bearing the same superscript do not differ significantly ($P = 0.05$).

phosphate even when the total phosphorus level of the diet was below the requirement of the chick. The phosphorus from hominy meal, which had promoted greater body weight gains than from corn meal, was less available for bone calcification than phosphorus from corn meal.

While these results are in contrast to the reports of many previous workers concerning plant phosphorus availability, it must be stressed that calcium and vitamin D_3 supplementation of the experimental diets was expressly designed to permit maximum usage of the organic phosphorus as developed from earlier studies by Vandepopuliere et al., (1961), Harms et al. (1962), and in previous chapters in this report. In addition, the organic phosphorus remained in its natural form in the plant material and was not chemically isolated.

Results from this study which indicate that organic plant phosphorus is highly available for growth are in agreement with the reports of Sieburth et al. (1952) and Temperton and Cassidy (1964 a). The observation that the organic phosphorus was less available for bone deposition confirms the report by Singsen et al. (1947) and Sieburth et al. (1952), but is in opposition to the findings of Temperton and Cassidy (1964 b).

It is apparent from the results of this study that the availability of the phosphorus in plant materials may be much greater than generally assumed. Variation in the phosphorus availability of different portions of the corn grain is indicated and may be responsible for some disagreement in results.

CHAPTER 10

SUMMARY AND CONCLUSIONS

A series of experiments were conducted to determine the possible effects of several factors upon the utilization of phosphorus by poultry. More than 17,520 chickens were used in these studies. The results of these experiments emphasized several factors which play a role in phosphorus metabolism and should be of value in guiding further research in this field of study. The results may be briefly summarized as follows:

Phosphorus Levels in the Maternal Diet

The addition of 0.35 percent phosphorus to hen diets containing 0.34 to 0.39 percent phosphorus resulted in significantly increased hatchability of fertile eggs. However, it did not affect the percentage of ash, calcium or phosphorus in the fresh egg or in the tibia of the day-old or 2-week-old chick.

The phosphorus content of the maternal diet did not affect the rate of mineralization of bones when chicks were fed a phosphorus supplemented diet. With regard to concentration of ash, phosphorus, or calcium in the tibia, there was as much variation between the chicks from the same hen as there was between chicks from different hens. Therefore, consideration of the phosphorus level of the maternal diet appears to be of limited value in the selection of chicks for the biological assay of phosphorus.

Variation Between Battery and Floor Conditions

The calcium and phosphorus requirements of chicks appear to be similar whether grown in batteries or in floor pens. Therefore, results obtained with chicks grown in batteries should be applicable to chicks grown in floor pens.

Comparison of Assay Techniques

Variation in the procedure used to assay phosphate sources or to interpret the results of such assays may significantly influence the relative biological value of a phosphate source. The use of different calcium:phosphorus ratios at different levels of phosphorus supplementation appears desirable in order to elicit maximum response of the chick and allow full utilization of the phosphorus.

Source of Calcium

No differences in calcium availability were observed between reagent calcium carbonate, reagent calcium sulfate, ground oyster shell, two samples of ground limestone, and reagent calcium gluconate when fed to chicks at levels ranging from 0.2 to 0.7 percent of the diet. Therefore, any of these sources could be used to supply the calcium in phosphorus assay diets without affecting performance of the chicks.

Vitamin D Levels

Levels of vitamin D_3 greater than those suggested by the National Research Council (1960) resulted in increased body weight and bone ash.

However, the response to increased levels of the vitamin become less as the calcium and phosphorus levels more closely approached the optimum. The response to vitamin D_3 supplementation appears to be due to enhancement of calcium absorption when dietary inbalances of calcium and phosphorus exist.

Availability of Phosphorus from Animal Protein Supplements

Phosphorus supplied to chicks from fish meal, poultry by-product meal, or meat and bone meal was utilized by chicks for body weight gains and bone calcification as well as dicalcium phosphate or monosodium phosphate. Therefore, no adjustment for availability of this mineral needs to be made in formulation of poultry diets when these animal protein supplements are used.

Availability of Phosphorus from Plant Sources

The phosphorus of phytic acid (inosital hexaphosphoric acid) was found to be highly available for growth and bone calcification of chicks. However, widening of the Ca:P ratio in the diet decreased the availability of the phosphorus in phytic acid to a greater extent than that from dicalcium phosphate.

Sodium phytate phosphorus was less available than phytic acid phosphorus, but the availability tended to increase as the level of supplementation increased. Calcium phytate phosphorus was relatively unavailable for growth purposes but somewhat more available for bone calcification. Increasing the vitamin D level of the diet from 360 to 1,080 I.C.U. per pound significantly increased the availability of phosphorus from calcium phytate but not from sodium phytate or phytic acid. Low calcium levels and narrow Ca:P ratios tended to improve the utilization of calcium phytate phosphorus. However, these low levels of calcium were not the most desirable levels for optimum performance on diets supplemented with dicalcium phosphate.

Phosphorus supplied to the chick from natural plant materials appears to be more available than generally assumed. Organic phosphorus from several plant feedstuffs promoted body weight gains that were equal or superior to inorganic phosphate supplements. However, this source of phosphorus was somewhat less available for bone calcification.

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BIOGRAPHICAL SKETCH

Park William Waldroup was born October 17, 1937, at Maryville, Tennessee. He was graduated from Midway High School, Kingston, Tennessee, in 1955 as valedictorian. He attended the University of Tennessee and received the Bachelor of Science degree in Agriculture in March, 1959, graduating first in his class. While attending this university he was awarded five scholarships, received the Chicago Tribune award for leadership, and was president of the National Collegiate Poultry Club. He was employed as a lab technician in the Poultry Department from September, 1955, to March, 1959.

In April, 1959, he accepted the position of Interim Assistant in Poultry Nutrition at the University of Florida. He received the degree of Master of Science in Agriculture in February, 1962.

From September of 1962 to June of 1964 he received a Ralston Purina Research Fellowship Award for studies toward the doctorate in Animal Science.

In June, 1964, he became a Research Assistant in the Poultry Science Department and was promoted to Research Associate in October, 1964.

He is a member of the Poultry Science Association, Alpha Zeta, Phi Kappa Phi, Gamma Sigma Delta, Phi Sigma, and Sigma Xi. He is an Elder and Sunday School Superintendent of Kanapaha Presbyterian Church of Gainesville.

He is married to the former Janet Ray of Crestview, Florida, and father of Mary Janet and Park Jr.

This dissertation was prepared under the direction of the co-chairmen of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment for the degree of Doctor of Philosophy.

April 25, 1965

M. A. Brooker
Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:

C. B. Ammerman
Co-chairman

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Co-chairman

Melvin Koger

R. L. Shirley

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